STRUCTURAL APPLICATIONS

OF

GAS PHASE ION-MOLECULE REACTIONS

BY

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TO

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AND

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(2) The Location of Olefinic Bonds in Mono- and Di-Unsaturated Compounds.
R.J. Greathead and K.R. Jennings.
Org. Mass Spectrom., 15, 8, 430-434 (August 1980).

(3) The Location of Olefinic Bonds in Mono- and Di-Unsaturated Compounds.
R.J. Greathead and K.R. Jennings.
British M.S. Conference, Canterbury, Kent (September 1980) (Oral).

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(5) Mixture Analysis Using Chemical Ionisation and Linked Scan Techniques.
R.J. Greathead and K.R. Jennings.
Royal Society of Chemistry, Perkin Division, N. Staffs. Poly.
(April 1981) (Oral).

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LIST OF ABBREVIATIONS

L.D.O.	- Locked Dipole Orientation
A.D.O.	- Average Dipole Orientation
E.I.	- Electron Impact
Q.E.T.	- Quasi-Equilibrium Theory
R.R.K.M.	- Rice, Ramsberger, Kassel and Marcus Theory.
C.I.	- Chemical Ionisation
P.C.I.	- Positive Chemical Ionisation
P.A.	- Proton Affinity
I.E.	- Ionisation Energy
N.C.I.	- Negative Chemical Ionisation
D.C.I.	- Desorption Chemical Ionisation
I.C.R.	- Ion Cyclotron Resonance
r.f.	- radio-frequency
C.I.D.	- Collision Induced Decomposition
C.A.	- Collisional Activation
M.I.K.E.S.	- Mass analysed Ion Kinetic Energy Spectrum
G.C.	- Gas Chromatograph
N.M.R.	- Nuclear Magnetic Resonance
V.M.E.	- Vinyl Methyl Ether
G.C./M.S.	- Gas Chromatography/Mass Spectrometry
I.R.	- Infra-red
U.V.	- Ultra-violet
M.I.	- Metastable Ion (Characteristics)
T	- Kinetic Energy (Release)
L	- Isotopic Labelling
H	- Heat of Formation
M.O.	- Molecular Orbital (Calculations)

C.S.	- Charge Stripping
F.I.K.	- Field Ionisation Kinetics
P.I.P.E.C.O.	- Photoion-Photoelectron Coincidence
P.E.	- Potential Energy (Surface)
D-labelling	- Deuterium labelling
GA	- Gibberellin
MS/MS	- Mass Spectrometry/Mass Spectrometry

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SUMMARY

A variety of mass spectrometers have been used to study four projects, involving three different applications of gas phase ion-molecule reactions. Firstly, the use of the vinyl methyl ether radical cation for the location of olefinic bonds (NEUTRAL STRUCTURE DETERMINATION) has been improved by altering the mode of production of the reagent ion. The scope and application of this technique have been reviewed. Secondly, the structures of various $C_3H_6O^{+\cdot}$ and $C_4H_5N^{+\cdot}$ ions (ION STRUCTURE DETERMINATION) have been studied. Reactions specific for the structures (i) $CH_3-O-CH=CH_2]^{+\cdot}$ and (ii) $CH_2=\overset{+}{O}-CH_2-\dot{C}H_2$ have been identified and employed to detect the presence of $C_3H_6O^{+\cdot}$ ions of these structures formed from a variety of precursors. These results provide an insight into the mechanisms of some gas phase elimination reactions and allow this method to be compared with those providing analogous information. Reactions have been sought to characterise the structure of $C_4H_5N^{+\cdot}$ ions generated from five different neutral precursors. Such reactions proved not to be identifiable readily, with the exception of a reaction involving 1.3-butadiene, which appears to be specific for the pyrrole molecular ion structure. Finally, the beginning of the application of the technique of Mass Spectrometry/Mass Spectrometry to the detection of gibberellins in mixtures (MIXTURE ANALYSIS) is described. The results of a survey of the chemical ionisation of the gibberellins are presented. The collision induced decomposition spectra of nine gibberellin/ammonium adduct ions are reported, showing that this method is capable of distinguishing readily all these gibberellins, some of which are isomers. Preliminary results involving crude grass extracts spiked with gibberellins are presented and methods of lowering the detection levels obtained have been suggested. Evidence concerning the pyrolysis of gibberellic acid is presented and discussed. These experiments and results serve to illustrate the varied use of gas phase ion-molecule reactions in structure determination.

CHAPTER 1

GAS PHASE ION-MOLECULE REACTIONS

1.1 INTRODUCTION

Chemical reactions between gaseous ions and neutral molecules were first discovered over sixty years ago,[1] yet it was not until three decades later that they began to be investigated systematically. In the 1950's the products of ion-molecule reactions, previously considered a nuisance, were studied by mass spectrometry.[2] Early research work was carried out using commercial mass spectrometers designed primarily for the low pressure electron impact ionisation analysis of organic compounds.[3] This pioneering work served to stimulate the design of new instrumental techniques which were more suited to the production of gas phase ion-molecule reactions and to the investigation of the physical and chemical nature of such reactions.[4] Over the last twenty years, advances in electronic and vacuum technology together with an increased awareness of the scope and applicability of gas phase ion-molecule reactions have lead to the rapid expansion of this subject.

1.2 SCOPE AND APPLICATION

The wide ranging interest in the subject of ion-molecule reactions may be witnessed by the publication over the last ten years of a variety of books[5-11] devoted entirely to this subject. Thus for the sake of clarity it is convenient to consider the subject of ion-molecule reactions from the physical and chemical viewpoints; although it is often difficult to separate the two. Several techniques such as Crossed /Merging Beams,[12] Tandem Mass Spectrometers,[13] Flowing Afterglow Reaction Tubes,[14] Selected Ion Flow Tubes,[14] High Pressure Pulsed Electron Beam Sources[15] and Ion Cyclotron Resonance[16,17] have been developed in order to obtain a better physical description of ion-molecule reactions in the gas phase. This has led to the experimental

determination of the thermal rate constants[18] and thermodynamic data[19] for a large number of ion-molecule reactions.

Concurrently, various modified Langevin collision theories[20] such as the Locked Dipole Orientation (L.D.O.), the Average Dipole Orientation (A.D.O.) and further refined A.D.O. theories have been developed in order to obtain a theoretical prediction of the collision rates of ion-molecule reactions and so derive a detailed descriptive fundamental model of the ion-molecule interaction. A measure of success has been achieved for the comparison of such theoretical collision rate constants with those obtained experimentally, especially for proton transfer reactions.[20,21] These ion-molecule reaction theories together with experimental data obtained from the above techniques have proved useful in elucidating the role of ion-molecule reactions in flames,[22] lasers,[23] electric discharges,[24] the upper atmosphere,[25] interstellar clouds,[26] radiation chemistry[7] and more recently in nuclear reactor cores.[27]

From the chemical viewpoint, improved designs for commerical Mass Spectrometers together with the development of the more specialised instrumental techniques, in particular Ion Cyclotron Resonance,[16,17] has led to a greater knowledge of the different types of chemical species that can be formed in the gas phase and to the postulation of chemical mechanisms by which they are so produced.[28] This synthetic gas phase ion chemistry has proved useful in elucidating the role of ions in solutions,[29] where solvent effects can often complicate the reactions, and in providing a greater understanding of the role of cluster ion formations in nucleation and solvation phenomena.[30] In general, ion-molecule reactions, such as proton transfer, have also proved useful for the trace analysis of a large number of compounds by a technique known as Chemical Ionisation Mass Spectrometry.[31]

1.3 SCOPE OF THIS THESIS

Work described in this thesis concentrates on the chemical aspects of gas phase ion-molecule reactions and in particular on the use of structurally specific reactions. Results are presented that show how the application of a specific ion-molecule reaction can be used to obtain structural information about either the neutral (Chapter 3) or the ionic (Chapters 4 and 5) species partaking in such a reaction. The work reported in Chapter 6 makes use of an ion-molecule reaction to affect a partial separation inside the mass spectrometer source through selective ionisation. This is used to aid the detection of specific components in crude mixtures by metastable ion monitoring.

CHAPTER 2

INSTRUMENTAL TECHNIQUES

2.1 MASS SPECTROMETRY

2.1.1 Introduction

A mass spectrometer[32-36] is an instrument based on the separation of gaseous ions according to their mass to charge ratios by some physical means. Its operation may be divided into four sequential stages. (1) Sample Introduction, (2) Ion Production, (3) Ion Separation and (4) Ion Detection. Each of these functions can be accomplished in a variety of ways. (A schematic view of a double focussing mass spectrometer is situated at the end of this section (2.1) to aid the understanding of these functions. (Fig. 2.1))

2.1.2 Sample Introduction

The method of sample introduction[37] employed depends upon three criteria: (a) volatility of the sample, (b) thermal stability, (c) whether one is dealing with a pure compound or a mixture. The design of sample inlet systems is restricted by three factors. Firstly, most mass spectrometers operate at pressures well below atmospheric pressure. Secondly, except for very specialised ionisation techniques, the sample must be volatilised prior to ionisation and thirdly the sources of magnetic sector instruments normally operate at high potentials. Therefore in general samples are introduced into the mass spectrometer ion source by means of a heated probe passing through a vacuum lock or from a heated reservoir via a leak or from a gas chromatograph via an appropriate interface.

2.1.3 Ion Production (Electron Impact)

The most common ionisation technique used in mass spectrometry is electron impact (E.I.). Electrons produced from an incandescent filament wire are accelerated by a potential difference through a small hole into

the ion source. The value of the potential difference, usually 70 electron volts (eV) (1 eV ≡ 96 kJ/mole) determines the (mean) energy of the electrons. The electron beam, collimated by the use of a small magnetic field, then interacts with the gaseous sample molecules (M), which are present in the ion chamber at pressures of 10^{-5}-10^{-6} Torr (1 Torr ≡ 133 N m^{-2}) to produce molecular radical cations ($M^{+\cdot}$) some, or all of which subsequently isomerise and/or fragment. The ionisation energy of most organic molecules lies in the range 7-13 eV and is defined as the energy required to move an electron from the highest occupied molecular orbital as represented by the equation

$$M + e \longrightarrow M^{+\cdot} + 2e \qquad 2.1$$

(Note that under these conditions the corresponding process of electron capture to produce an anionic species is less favourable by a factor of 10^{-2}). For many compounds the structure of $M^{+\cdot}$ closely resembles that of M and so the structure of the neutral may be inferred from the observed fragment ions.

Several theories, notably the Quasi-Equilibrium Theory[38] (Q.E.T.) and the Rice, Ramsberger, Kassel and Marcus theory[39] (R.R.K.M.) have been developed to provide a mathematical description of the ionic fragmentations occurring in the ion source. Whilst the detailed mathematics of these theories will not be discussed, it is necessary to consider the fundamental basis of these theories in order to assess the technique of electron impact.

It takes an electron (50 eV), approximately 10^{-16} seconds, to traverse a molecule with a diameter of the order of a nanometre. This is about 10^{2} times faster than the fastest vibration, a C-H stretch. Thus ionisation by electron impact follows the Frank-Condon principle which requires that the position and momenta of the nuclei

do not change during the transition. The energy of the ionising electron is usually higher than the ionisation energy of the molecule and so in addition to the thermal energy present in the molecule excitation energy may be transferred to the molecule during ionisation. The low (source) pressure of sample molecules and short source residence time (10^{-5}-10^{-6} s) precludes any ion-molecule collisions in the ion source. This means that there is no collisional energisation/deactivation mechanism to effect the redistribution of excitation energy in the ions and therefore this distribution is non-Boltzmann and an average temperature of the ions cannot be assigned.

Transitions to various electronic, vibrational and rotational states are possible during ionisation leading to a collection of ions with a wide distribution of internal energy. Experimental evidence, particularly that of metastable ions and field ionisation, shows that fragmentation of molecular ions is not spontaneous but occurs after a redistribution of the excitation energy. This redistribution occurs mainly by radiationless transitions involving vibrational relaxation, vibronic relaxation and isomerisation. Various systems, for example $C_4H_8^{+\cdot}$,[40] $C_6F_6H_2D_2^{+\cdot}$,[41] have been shown to exist where complete energy randomisation prior to decomposition does <u>not</u> occur; however a large majority of ions seem to decompose after complete randomisation.

After energy randomisation, fragmentation occurs by unimolecular decomposition of the molecular ions through consecutive, competitive losses of neutral and/or radical species. During these decompositions the specific amount of internal energy and angular momentum with which the molecular ion was formed are conserved independently.

2.1.4 *Ion Separation*

Ions leave the ion source either by free diffusion or through the application of electric fields and are subsequently focussed into the analyser region where they are physically separated according to their mass (m) to charge (z) ratios. Various analysers,[32-34] usually employing the application of variable electric and/or magnetic fields, have been employed to effect ion separation, for example, magnetic deflection, time of flight, quadrupole lens, radio frequency, cyclotron resonance and cyclod al focussing. Work described in this thesis has been performed using an ion cyclotron resonance spectrometer, the principles of which are described in Section 2.3, and several double focussing mass spectrometers.

The mass separation technique utilised in a double focussing mass spectrometer is that of magnetic deflection. The magnetic field (B) is used to focus ions which have been subjected to a fixed accelerating voltage (V). Ions describe a circular path through the magnetic field according to the equation

$$m/z = B^2 r_b^2 / 2V \qquad 2.2$$

where r_b is the radius of the magnet. Ions can be brought to focus at the end of the magnetic sector and a mass spectrum is obtained either by scanning V at fixed B or as is more usually the case by scanning B at fixed V.

One of the problems of using only this one sector is that the ability to separate ions of different masses (resolving power) is limited by the initial high spread of translational energies of the ions leaving the source. This cannot be corrected in a single focussing magnetic sector instrument because the magnetic analyser is direction focussing and <u>not</u> velocity focussing. To overcome this

problem an electrostatic analyser[33] is employed. Ions are deflected by a radial electrostatic field such that their circular trajectories are described by the equation

$$r_e = 2V/E \qquad 2.3$$

where r_e is the radius of the electric sector and E is the value of the electrostatic field (V as before). Note that m is not a parameter in this equation and so the electrostatic analyser does not mass analyse. However, V is a parameter and so this sector is an energy analyser. Thus by the appropriate combination of electric and magnetic sectors a directionally focussed ion beam of given m/z homogeneous in energy may be obtained. If a direction focussing electric sector is followed by a direction focussing magnetic sector then simultaneous direction and velocity focussing of the ion beam can be obtained. The general criterion for this to be true is

$$r_e = Kr_b \qquad 2.4$$

Several double focussing mass spectrometers have been developed whereby the magnetic sector precedes the electric sector.[42] As will be seen later such "reverse geometry" mass spectrometers offer certain advantages for the investigation of metastable ions.

2.1.5 Ion Detection

With the exception of ion detection in an ion cyclotron resonance spectrometer, which will be discussed later, two general methods of ion detection[32] are employed in mass spectrometry. The first method involves the simultaneous focussing of all the ions in a plane to be recorded on a photographic plate. This is a sensitive method allowing the detection of all the ion currents at all times and allowing intensity and sensitivity measurements to be made with ease. The

necessity of development techniques followed by the use of a densitometer make its operation cumbersome. The second method which has been used extensively in this work allows the separated ions of different m/z ratios to pass sequentially through a slit and strike a collector. The collector produces a flow of electrons which is proportional to the individual ion currents. Amplification of the ion signal by for example an electron multiplier or a scintillator/photomultiplier in the case of the Daly detector[43] makes it possible to detect single ions arriving at the collector. The accuracy and sensitivity of electronic detection systems means that although the efficiency of ionisation and transmission is very poor and may only yield one ion at the collector per 10^6 sample molecules in the ion source, excellent spectra can be obtained from submicrogram quantities. When electrical detection is employed the amplified signal is passed to a computer via an analogue-to-digital converter or to a chart recorder. The mass spectrum is thus obtained by scanning the magnetic field whilst recording the individual collected ion currents.

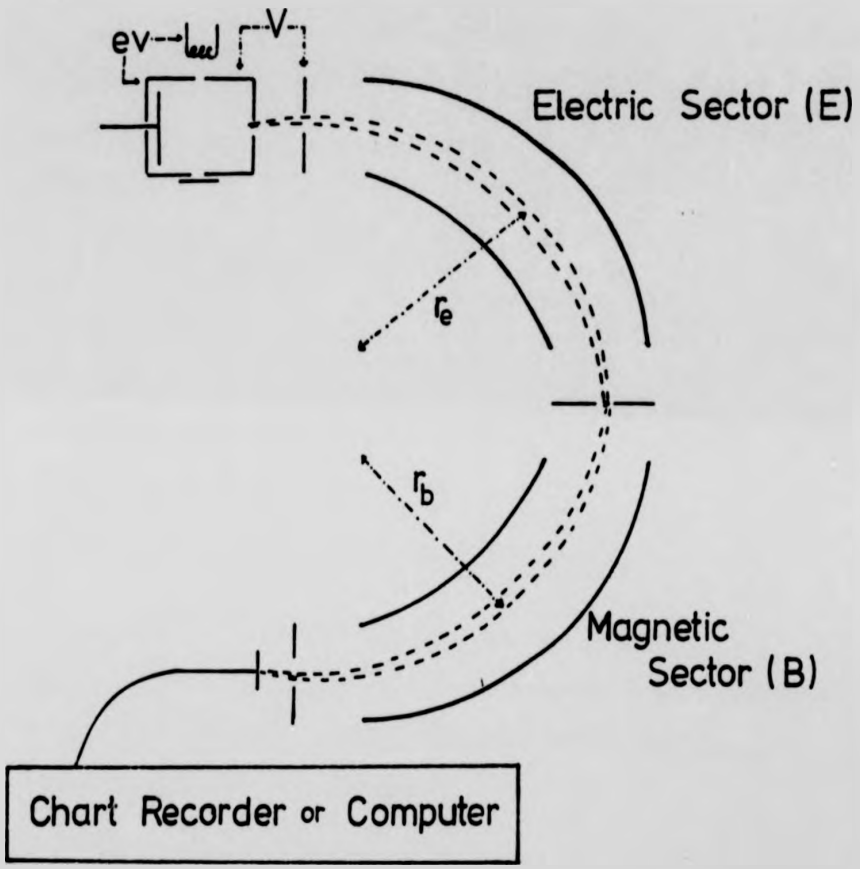

Figure 2.1. Schematic of a Nier-Johnson double focussing mass spectrometer.

2.2 ALTERNATIVE IONISATION TECHNIQUES

2.2.1 Introduction

Although electron impact serves as a good general ionisation technique it suffers from certain specific disadvantages. Most of these arise from the unknown high amount of energy deposited in the molecule upon ionisation as discussed earlier.

(A) Unstable Molecular Ions

For certain classes of compounds, $M^{+\cdot}$ is unstable with respect to dissociation even at low internal energies. In such cases almost all $M^{+\cdot}$ ions fragment in the source with subsequent loss of high mass and molecular weight information.

(B) Isomerisation Versus Decomposition

Because the rate of decomposition of ions is slow compared with that for energy randomisation, ions may isomerise prior to decomposition. This is not in general a problem, although isomeric samples often yield identical electron impact spectra.

(C) Stereochemistry

Again, because of energy randomisation prior to decomposition, stereochemical information may be lost if electron impact is used to produce the ions.

(D) Internal Energy Distribution

The exact internal energy distribution of the ions produced by electron impact is generally unknown.

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(E) Involatile and Thermally Unstable Compounds

Due to the prerequisite of sample vaporisation, compounds which either do not easily vaporise or decompose upon vaporisation cannot be fully analysed by electron impact.

Some of these problems have been overcome by a variety of alternative methods of ionisation. These include Chemical Ionisation;[31] Field Ionisation/Desorption,[44] Californium 252 Plasma Desorption,[45] Direct Laser Ionisation,[46] Secondary Ion Bombardment[47] and Fast Atom Bombardment.[48] Over the past ten years only chemical ionisation and field ionisation/desorption have been developed for organic analytical work to the stage where they are widely available as basic instrumentation. Fast atom bombardment however shows great promise as an ionisation technique of the future for thermally labile and unstable compounds.

2.2.2 Chemical Ionisation

The technique of chemical ionisation (C.I.) first described by Munson and Field[49] in 1966, has been reviewed regularly over the last twelve years,[50] the most recent review being by Jennings.[31]

By analogy with electron impact, the technique of chemical ionisation utilises a collision between an ion (reagent ion) and a sample molecule (M) to form product ions which are characteristic of the sample molecule. Experimentally chemical ionisation is achieved by bombarding a reagent gas or mixture of gases in an ion source at a pressure of approximately 1 Torr with a beam of high energy electrons (typically 150-400 eV) to produce a plasma of ions, some of which, reagent ions, will be present at a high concentration. The reagent ion(s) may be a molecular ion, a fragment ion or the product of an ion-molecule reaction between a primary reagent gas ion and a reagent gas molecule which is stable with respect to further reaction with

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reagent gas molecules. Sample molecules are introduced into the ion source in a low concentration (approximately 0.1%) relative to the reagent gas. Under these conditions very few sample molecules will be ionised directly by electron impact, but reagent ions may undergo ion-molecule reactions with the sample molecules. The product ions of such reactions, together with those formed by subsequent fragmentations, constitute a chemical ionisation mass spectrum of the sample.

Unlike ionisation by electron impact, the ionisation process cannot be thought of in terms of a Frank-Condon process, but rather in terms of a slow equilibrium adjustment of electronic states and atomic positions as the two particles form a collision complex, the life-time of which is long compared with rotational and vibrational periods. The collision complex thus loses knowledge of its mode of formation and the excess internal energy is randomised among the internal degrees of freedom. The fragmentation pathways are then characterised by the energy content of the complex, as in the quasi-equilibrium theory. Under the high pressure ion source conditions thermal equilibrium is attained through ion-molecule collisions so that chemical ionisation provides a means of studying the kinetics and thermodynamics of gas phase ion-molecule reactions in the absence of solvent effects.

Another difference between chemical ionisation mass spectrometry and electron impact mass spectrometry is that most of the commonly used reagent gases generate even electron ions, for example MH^+, $(M-H)^+$, $(M-H)^-$, $(M+Cl)^-$, the fragmentations of which are invariably simpler than those of the odd electron ions produced by electron impact. Charge localisation can be used to predict the fragmentation of a molecular ion produced by electron impact by assuming that the charge is localised at a specific site in the ion. By analogy, it is often possible to rationalise the fragment ions produced under

chemical ionisation conditions by the assumption that the reagent ion reacts with the sample molecule at a particular site. Indeed, such an approach can be shown to be useful for determining the location of a specific functional group within a molecule (see Chapter 3).

Chemical ionisation has been termed a "soft" ionisation technique because it is less energetic than electron impact. Furthermore both the energetics of the reaction and the type of chemistry occurring can be more finely controlled by the careful choice of the reagent ion employed.

2.2.3 *Positive Chemical Ionisation (P.C.I.)*

Proton transfer has become the most widely used ion-molecule reaction in chemical ionisation mass spectrometry because of the general high efficiency of such reactions. A proton transfer reaction, which can be thought of in terms of a Bronsted acid (AH^+)/Brönsted base (M) reaction, will be exothermic provided that the proton affinity of M is greater than that of A;[51] as represented by equations 2.5 and 2.6, where P.A.(X) is the proton affinity of X.

$$M + AH^+ \longrightarrow MH^+ + A \qquad 2.5$$

$$\Delta H = P.A.(A) - P.A.(M) \qquad 2.6$$

For a particular sample molecule, the magnitude of the exothermicity of this reaction (ΔH) can be changed by altering the gas phase acid/reagent ion being used. As the exothermicity of the reaction lies mainly in the newly formed bond ($M\text{-}H^+$) as vibrational energy then the use of a strong acid like H_3^+ will lead to a considerable amount of fragmentation of MH^+ compared with the use of a weaker acid like $C_4H_9^+$. Table 2.1 lists the most commonly used reagent gases together with the major reagent ion (AH^+) that is formed in each reagent gas in increasing order of the

proton affinity of A and decreasing order of the fragmentation of MH^+.

Table 2.1. Commonly Used Positive Chemical Ionisation Reagent Gases.

Reagent Gas	Major Reagent Ion (AH^+)	Proton Affinity[51] P.A.(A) kJ mol^{-1}
Hydrogen	H_3^+	535
Methane	CH_5^+	706
Isobutane	$C_4H_9^+$	755[a]
Ammonia	NH_4^+	840
Dimethylamine	$(CH_3)_2NH_2^+$	912

[a] Note that A is taken to be 2-methylpropene.

Although the formation of MH^+ is usually the major reaction when methane, isobutane and ammonia are used as reagent gases peaks due to addition reactions may also be observed under appropriate conditions. In methane low intensity $(M+C_2H_5)^+$ and $(M+C_3H_5)^+$ ions may be observed and in isobutane $(M+C_3H_7)^+$ and $(M+C_4H_9)^+$ ions may be observed. The proton affinities of most oxygen containing organic compounds fall in the range 710–830 kJ mol^{-1}.[51] Proton attachment to such compounds by NH_4^+ is therefore endothermic, although for many compounds NH_4^+ attachment to give $(M+NH_4)^+$ is exothermic. Therefore as a general rule, NH_4^+ will protonate compounds containing nitrogen such as amines and will form attachment ions $(M+NH_4)^+$ with oxygen containing compounds such as sugars.

2.2.4 *Charge Transfer (Exchange)*

Charge transfer is a specialised form of chemical ionisation whereby a molecular reagent ion ($X^{+\cdot}$) transfers its charge to (or removes an electron from) a sample molecule (M) to produce an excited molecular

ion ($M^{+\cdot}$), the internal energy of which is determined by the difference in ionisation energies of X and M; as represented by equations 2.7 and 2.8 where I.E.(X) is the ionisation energy of X.

$$X^{+\cdot} + M \longrightarrow M^{+\cdot} + X \qquad 2.7$$

$$\Delta H = \text{I.E.(M)} - \text{I.E.(X)} \qquad 2.8$$

Nitrogen and argon, for which the ionisation energies are 15.6 eV[52] and 15.8 eV[52] respectively, are the most commonly used reagent gases of this type. Because they give rise to the odd-electron ions ($M^{+\cdot}$) they give spectra which are not dissimilar to electron impact spectra, except that the intensity of the molecular ion peak is usually much smaller in the case of charge transfer since no molecular ions with low internal energy are formed. The abundance of $M^{+\cdot}$ can be increased by using a reagent ion with a lower ionisation energy, for example $NO^{+\cdot}$ from an N_2/NO mixture. (Ionisation energy NO = 9.3 eV.[52]) Care must be taken that the reagent ion does not react other than by charge exchange with the sample molecules.

2.2.5 *Negative Chemical Ionisation (N.C.I.)*

One of the main mechanisms for excess internal energy loss from a negative ion is by electron detachment with concomitant loss of charge. This places more stringent requirements upon the mode of formation of negative ions compared with that of positive ions.

There are three methods of negative ion production by electron impact. These are:-

(1) Resonance Capture or Three Body Attachment

$$AB + e\ (+M) \longrightarrow AB^{-}\ (+M) \qquad 2.9$$

(2) Dissociative Resonance Capture

$$AB + e \longrightarrow A^- + B^{\cdot} \qquad 2.10$$

(3) Ion Pair Production

$$AB + e \longrightarrow A^- + B^+ + e \qquad 2.11$$

Processes (1), (2) and (3) occur at electron energies of 0-10 eV, 0-15 eV and > 10 eV respectively. Thus under normal electron impact conditions relatively few negative ions are formed because the low population of low energy (thermal) electrons precludes the formation of low internal energy stable ions by electron capture. However, under high pressure chemical ionisation conditions the use of a buffer gas such as nitrogen, methane or isobutane facilitates the thermalisation of electrons and thus allows compounds with a positive electron affinity to form molecular anions by electron capture.

If a stable molecular ion cannot be formed then a reaction between a negative reagent ion and a sample molecule may be utilised. Various halogenated compounds can be used to produce X^- ions (X = F, Cl, Br or I) which will react with a variety of compounds to give a stable adduct ion of the form $(M+X)^-$. By analogy with positive chemical ionisation mass spectrometry, reagent ions that react as Brönsted bases can be used to abstract a proton from a sample molecule.

$$X^- + M \longrightarrow (M - H)^- + H X \qquad 2.12$$

$$\Delta H = P.A.(M - H^-) - P.A.(X^-) \qquad 2.13$$

A few examples of such Brönsted bases are given below.

X = H^-, OH^-, CH_3O^-, C_2H^-, SH^-, CH_3COO^-, $C_6H_5COO^-$

P.A.(X^-)[53] = 1673, 1632, 1577, 1561, 1468, 1443, 1406 kJ mol^{-1}

OH^- has proved to be the most generally useful reagent ion of this type. $O_2^{-\cdot}$ and $O^{-\cdot}$ have been shown to react with a wide range of compounds in a variety of different ways, including proton abstraction. The problem of short filament life in the presence of high pressures of oxygen has been alleviated by producing $O_2^{-\cdot}$ and $O^{-\cdot}$ from mixtures of reagent gases or by passing oxygen through a Townsend discharge.[54]

Because much of the exothermicity of negative ion-molecule reactions occurs as vibrational energy in the newly formed bond in the neutral species then the newly formed ion is usually very stable with respect to dissociation and loss of charge. The general lack of fragmentation exhibited in negative chemical ionisation means that most of the ion current is carried by one or two structurally significant high mass ions. This fact coupled with the high sensitivity, two to three orders of magnitude higher than positive E.I./C.I. for compounds with a high electron affinity, makes this a powerful technique for low level detection by single ion monitoring of electronegative compounds in the environment. Such applications of negative ions together with the fundamental process of negative ion production have been reviewed.[53,55]

2.2.6 *Desorption Chemical Ionisation*

Desorption chemical ionisation (D.C.I.) reviewed recently by Cotter,[56] has received considerable attention over the last few years as a method of obtaining chemical ionisation spectra of involatile and thermally unstable compounds. The basic philosophy behind the technique is that instead of placing the sample one to two centimetres outside the ion source (Fig.2.2(a)) the sample is introduced directly into the ion source by means of an extended probe (Fig. 2.2(b)).

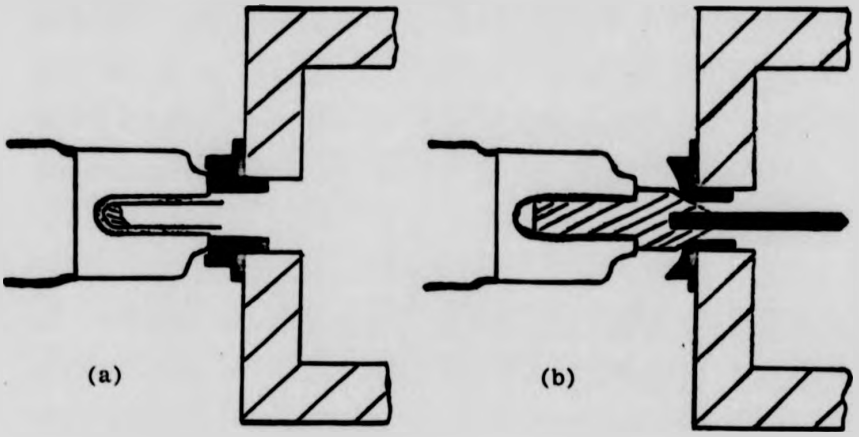


Figure 2.2. Comparison of solid sample introduction techniques.
(a) Glass capillary method. (b) Direct method.

There has been a slight controversy over the exact nature of the ionisation process[55] but there is no doubt that introducing the sample directly into the reagent ion plasma offers several advantages. Firstly it reduces the amount of time between sample vaporisation and ionisation and so reduces pyrolysis problems. Secondly it provides a better seal for the ion source and so improves the stability and reproducibility of the pressure therein. The major disadvantages of the technique are that the sample tends to be lost in a short time and the design of heated probes for use with high voltage sources is not easy. Thus the technique is ideally suited to a quadrupole mass spectrometer/data system which has the advantages of low source voltage and fast scanning but the limitations of low mass range and low resolution. However, recent advances in laminated magnets and probe heater designs now make the technique more applicable to the use of large magnetic sector instruments. Further work using inert matrices may lead to a method whereby the sample is released more slowly.

A home-built D.C.I. probe designed by Dr. R.S. Mason has been

used for the work described in Chapter 6. The probe consists of a piece of tungsten rod (21 x 0.75 mm) held on the end of a normal solids probe tip by means of a piece of machined teflon. The reagent gas and source heater are used to heat the sample.

2.2.7 *Field Ionisation*

Although field ionisation[44] has not been used in the work described in this thesis the technique has become well established and as will be seen in Chapter 3, can provide a viable alternative approach to chemical ionisation. In a field ionisation source, ions are produced from the sample in the gas phase at an emitter (anode), which may be a wire or a blade edge, close to a slit (cathode) in an electric field of the order of 10^7-10^8 V/cm. The field distorts the barrier between electrons in the occupied orbitals of the sample molecules near the anode surface and vacant conduction bands in the metal surface. Electrons are transferred from the sample molecules, producing ions which are accelerated by the field through the slit. The wide divergent ion beam is then focussed into a mass spectrometer. In spite of the high emitter ion currents obtainable the intensity of the ion current at the detector is smaller by a factor of approximately 100 compared with ionisation by electron impact due to the wide divergence of the ion beam after acceleration through the slit.

Although the ions are formed in the ground states and thus little decomposition occurs, fragmentation can occur by two mechanisms:-

(1) Field dissociation due to distortion of the energies of bonding orbitals by the high field.

(2) Thermal decomposition due to the thermal energy of the original molecule, if such energy is available.

There are two main advantages of field ionisation. Firstly field ionisation spectra have an enhanced molecular ion compared with electron impact spectra because the molecular ions are formed with little excess energy. Secondly the short source residence time (10^{-14}-10^{-12} s) means that usually no fragment ions generated by rearrangement or multiple decompositions are observed. Thus it is possible to obtain fragmentation information prior to isomerisation.

The possibility of observing the fragmentation and isomerisation of ions in the picosecond time frame, known as Field Ionisation Kinetics[56] and the development of Field Desorption[44] for the mass spectrometric analysis of involatile and thermally unstable compounds have led to the establishment of field ionisation as a major alternative ionisation technique.

2.3 ION CYCLOTRON RESONANCE (I.C.R.)

2.3.1 Introduction

Ion cyclotron resonance,[16,17] a widely used technique for the investigation of low pressure (10^{-6}-10^{-4} Torr) ion-molecule reactions, is based upon the following principles. Ions in a magnetic field describe a circular path. For a given magnetic field strength the angular velocity and hence cycloidal frequency of a particular ion is dependent upon its mass to charge ratio. Detection of an ion is made possible by the application of an electrical radio-frequency (r.f.) field. When the frequency of the applied field is equal to that of the ion motion then energy can be transferred to the ion which is then said to be in resonance.

The I.C.R. principle was first applied by Hipple[58] in 1949 in the form of an omegatron. Wobschall[59] developed the first I.C.R. spectrometer for studying ion-molecule reactions and the first commercial instruments became available in 1966 (Varian Associates).[60] Since then the principles of I.C.R. together with its physical and chemical applications have been well reviewed.[16,17,61]

The main advantage of the I.C.R. technique for the investigation of ion-molecule reactions is the facility of double resonance which allows the reactant/product ion relationship to be determined. However, I.C.R. suffers from the disadvantages of low resolution and a limited mass range (200 daltons). In order to try and overcome these and other limitations various techniques have been developed recently, for example Rapid Scan I.C.R.,[62] Fourier Transform I.C.R.[63]

2.3.2 *Ion Motion*

Ions of mass m and charge z which are moving in a plane normal to a uniform magnetic field B with a velocity v, experience a force F,

$$F = zvB \qquad 2.14$$

which is normal to both B and v. This results in a circular motion for which the acceleration, a, is equal to v^2/r where r is the radius of the ion path. Hence,

$$F = ma = mv^2/r = zvB \qquad 2.15$$

If ω_c is the angular frequency of the ion in rad s^{-1}, then

$$mv/r = m\omega_c = zB \qquad 2.16$$

or

$$\omega_c = zB/m \qquad 2.17$$

Equation 2.17 shows that the angular frequency of an ion is dependent upon its mass to charge ratio (m/z) and the strength of the magnetic field B; but independent of its velocity (v). It should be noted that the cyclotron frequency of an ion (ω_c) is more conveniently expressed in units of KHz.

$$\nu_c = \frac{\omega_c}{2\pi} \text{ KHz} \qquad 2.18$$

If a static electric field (E) is applied normal to the magnetic field (B) then a drift motion (v_d)

$$v_d = E/B \qquad 2.19$$

is superimposed upon the circular motion of the ions in a direction that is normal to both B and E. Equation 2.19 shows that all ions have the same drift velocity for constant electric (E) and magnetic (B) fields.

In the absence of an initial velocity the resultant ion path is a common cycloid as shown in Figure 2.3. Since the ions will have a thermal velocity in any possible direction then various cycloidal paths are possible.

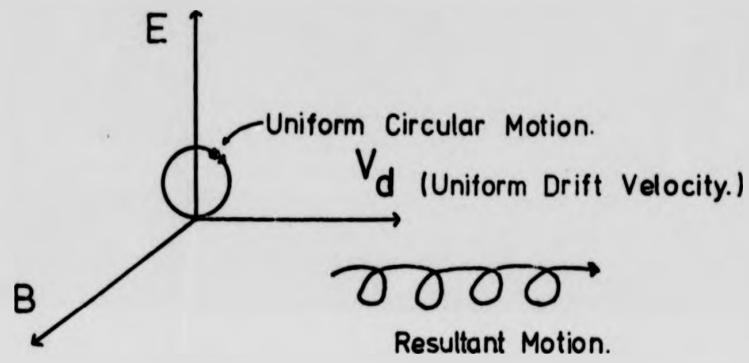


Figure 2.3. The two components of the motion of a positive charge in crossed electric and magnetic fields.

2.3.3 *Instrumentation*

The heart of an I.C.R. instrument is the ion cell (Fig. 2.4) which is mounted in a vacuum system between the poles of an electromagnet. Sample molecules which have been introduced into the vacuum system from a reservoir via a manual leak valve are ionised by electron impact. The ionising electrons are emitted from a hot wire filament and are collimated by a potential difference (electron voltage) between the filament and the cell and by the magnetic field. The electron current passing through the cell and falling on to the trap plate is measured and used to regulate the electron emission from the filament. In a drift cell, ions move through the cell in a cycloidal path, from the source region towards the ion collector region, under the influence of crossed magnetic and electric fields. The electric field results from an applied voltage between the drift plates of the cell. Because the motion of the ions in the direction of the magnetic

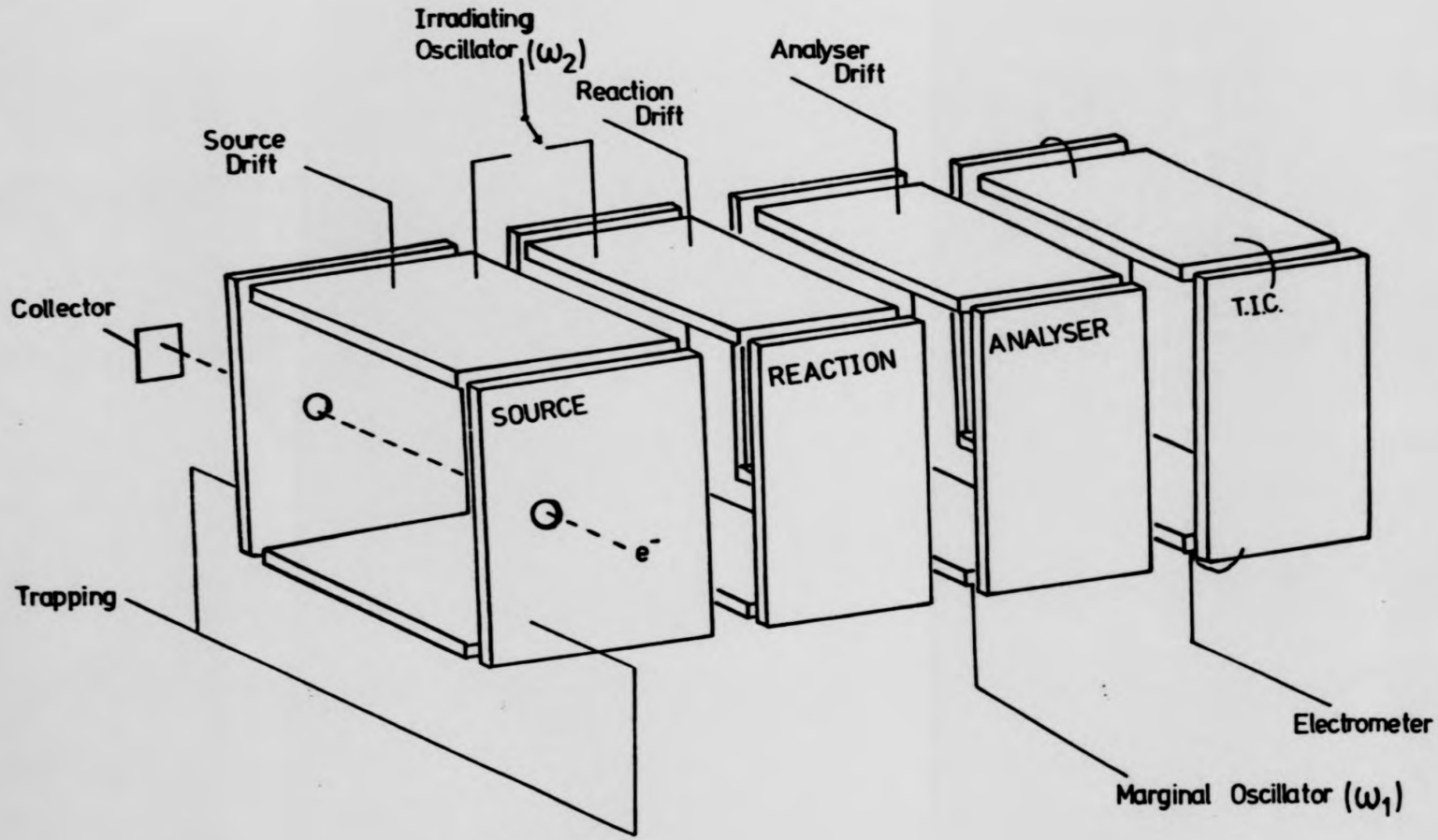


Figure 2.4. Schematic of a 4-section I.C.R. drift cell.

Table 2.2. Typical Operating Conditions and Dimensions of a "Square" Drift Cell.

Dimension of the cell	2.54 x 2.54 x 12.5 cm
Separation of the drift plates	2.54 cm
Operating Pressure Range	2×10^{-6}-6×10^{-5} Torr
Background Pressure	$\leq 1 \times 10^{-6}$ Torr
Trapping Voltage	$\leq$ 0.5 V
Drift Voltage	~ 0.5 V
Ionising Energy	11-15 eV
Trap Current	50-100 nA
TIC (Keithley 602 electrometer)	10^{-12}-10^{-11} A

For CO_2^+ + m/z = 44, magnetic field B = 10 K gauss

$$\nu_c = \frac{\omega_c}{2\pi} = 350 \text{ KHz}$$

with $E = 0.2 \text{ V cm}^{-1}$, $v_d = 3 \times 10^3 \text{ cm s}^{-1}$

T (time spent in the cell by the ion) = 2.5×10^{-3} s

D (diameter of the cyclotron orbit of the ion) = 0.4 mm

d (distance of drift during one revolution) = 0.11 mm

Distance travelled by ion = 0.8 m

field is not defined, a small voltage (less than 2 volts) is applied to the trapping (side) plates in order to keep the ions in the centre of the cell. The side, top, bottom and end plates in the ion collector region are all connected at ground potential. The total ion current, which is usually only a few pA is measured by an electrometer. A summary of typical operating conditions used in the I.C.R. spectrometer at Warwick (Varian Associates V-5900 I.C.R.) is given in Table 2.2.

Rather than use the high pressures of a chemical ionisation source to produce ion-molecule reactions, I.C.R. exploits the cycloidal motion of the ions. This motion provides a long path length (~ 1 m) and a long ion lifetime (~ 10^{-3} s) and consequently ion-molecule reactions can occur at comparatively low pressures (10^{-6}-10^{-4} Torr). Low energy electrons (10-15 eV) are generally used so as not to encourage the formation of fragment ions and to reduce the possibility of isomerisation. The internal energy distribution of the ions is non-Boltzmann and since no high source voltages are used the ions have low translational energies.

2.3.4 *Ion Detection (Single Resonance)*

As derived in Section 2.3.2 the cycloidal motion of an ion has a characteristic angular frequency (ω_c) given by equation 2.17

$$\omega_c = z\frac{B}{m} \qquad 2.17$$

If an r.f. oscillator of frequency ω_1 is applied to the drift plates of the analyser region of an I.C.R. cell (Fig. 2.4), that is perpendicular to B, then absorption of energy by the ions occurs when $\omega_1 = \omega_c$. The detection of the energy absorbed by the ions is facilitated by the use of a radio frequency marginal oscillator[64] to supply ω_1. The analyser drift plates of the cell are incorporated into the marginal oscillator

circuit and act as a capacitance in a tuned circuit. If the capacitance of the cell is altered by ions absorbing energy then the quality (Q) value of the circuit will be altered. Since $V \propto Q$ where V is the amplitude of the output signal and $Q = W/W_d$ where W is the total energy stored in the circuit and W_d is the energy dissipated per cycle then,

$$\frac{\Delta V}{V} = \frac{\Delta Q}{Q} = \frac{W_d^*}{W_d + W_d^*} \qquad 2.20$$

where W_d^* is the energy absorbed by the resonant ions. Thus the variation in the output amplitude gives a measure of the energy absorbed by the ions. A marginal oscillator has a high Q value. This results in a high voltage output magnification and a response over a narrow frequency range thus aiding sensitivity and resolution.

The absorption of energy by an ion causes the velocity and hence the radius of the cycloidal path to increase. Thus as the ions are brought into resonance either they will be ejected from the cell in the analyser region or else they will be discharged in the ion collector region. See Figure 2.5.

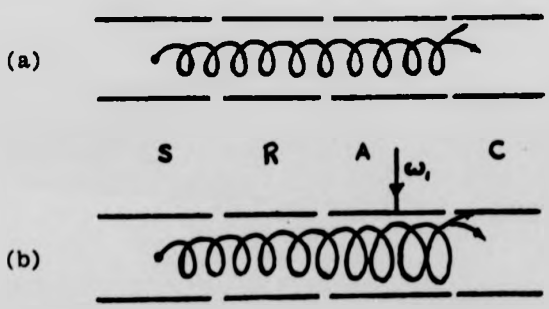


Figure 2.5. Schematic view of ion motion in the I.C.R. cell;
(a) ion motion in the absence of an r.f. field
(b) ion motion when the r.f. field (ω_1) is equal to the cyclotron frequency of the ion (ω_c).
S = source region, R = reaction region,
A = analyser region and C = collector region.

A mass spectrum can be obtained either by scanning the frequency (ω_1) of the r.f. field at constant magnetic field or by scanning the magnetic field (B) at constant r.f. oscillator frequency. In practise the latter method is used in conjunction with marginal oscillator detectors because it presents less difficulty.

The absorption of energy by an ion in resonance is monitored by signal modulation[16] and phase sensitive detection[65] in order to enhance the signal to noise ratio. Either some resonance condition or the supply of ions is interrupted at a given frequency, usually 27 Hz, and a phase sensitive detector compares the signals obtained. A variety of modulation techniques such as trapping modulation, electron energy modulation, emission current modulation and field modulation have been employed[16] with the last named technique being used exclusively in this work. Figure 2.6 illustrates the principle of field modulation.

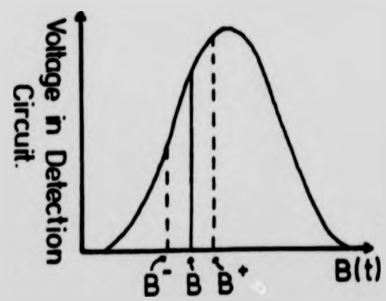


Figure 2.6. Schematic illustrating field modulation.

The magnetic field is modulated over a range of typically 20 gauss. B(t) sweeps across the absorption curve. Helmholtz coils which are placed just inside the magnetic pole caps repeatedly impose a small incremental field B^+ followed by an equal decremental field B^-, on B(t). The phase sensitive detector compares the signal at B^+ with that obtained at B^-. As B sweeps through the curve, a derivative

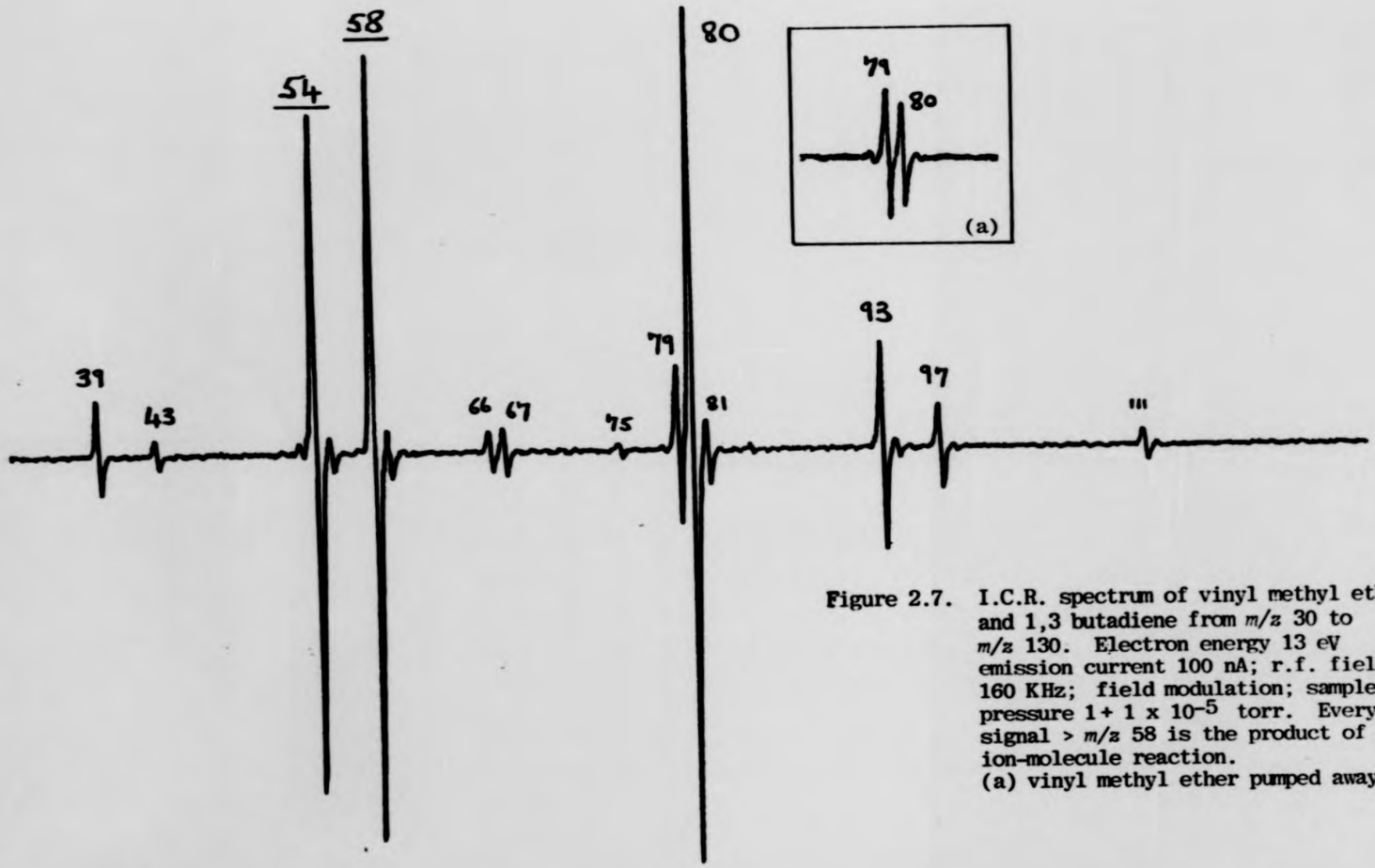


Figure 2.7. I.C.R. spectrum of vinyl methyl ether and 1,3 butadiene from *m/z* 30 to *m/z* 130. Electron energy 13 eV emission current 100 nA; r.f. field 160 KHz; field modulation; sample pressure 1+ 1 x 10^{-5} torr. Every signal > *m/z* 58 is the product of an ion-molecule reaction.
(a) vinyl methyl ether pumped away.

trace is thus produced. A typical single resonance spectrum is shown in Figure 2.7.

2.3.5 *Double Resonance*

The availability of various double resonance and ion ejection techniques facilitates the elucidation of the mechanisms of the ion-molecule reactions occurring in the I.C.R. spectrometer.[66] The rate constant (k) of an ion-molecule reaction

$$R^+ + N \xrightarrow{k} P^+ + F \qquad 2.21$$

depends on the translational energy of the reactant ions (R^+). This can be used to establish a connection between the reactant ions (R^+) and the product ions (P^+) in the following way. The magnetic field is adjusted until the reactant ions (R^+) are in resonance for a given observing oscillator frequency (ω_1) and the signal intensity of P^+ is monitored. A second, variable electrical oscillator (ω_2) is applied to drift plates of either the source or the reaction region (Fig. 2.4). This irradiating or double resonance oscillator is scanned over a range of frequencies in order to cover the mass range of the other ions in the cell. When ω_2 is equal to the cyclotron frequency of R^+ these reactant ions will absorb energy with a subsequent increase in their velocity. This results in a change in the translational energy of the reactant ions causing a corresponding change in the intensity of the product ions (P^+) which is being monitored. If the reaction is exothermic this will give rise to a negative double resonance signal .

Field modulation cannot be employed during double resonance experiments because of the requirement of a fixed magnetic field. This problem is overcome by pulsing the double resonance oscillator as shown in Figure 2.8.

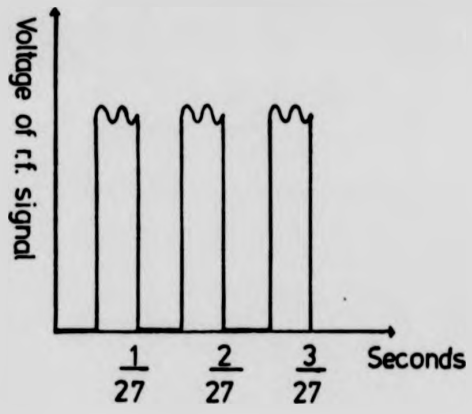


Figure 2.8. Scheme illustrating pulsed double resonance modulation.

It is important to differentiate between "threshold" and "sweep-out" double resonance experiments. If the second irradiating oscillator (ω_2) has a sufficiently large amplitude then the reactant ions will absorb enough energy to contact the upper or lower plates of the cell and so be swept out (see Figure 2.7). This process can usually be recognised by monitoring the total ion current whilst performing the double resonance experiment.[67] The sweeping out of a large number of ions can give rise to a spurious double resonance signal[67] due to either a de-tuning effect or a space charge effect and can also reverse the sign of a positive double resonance signal. The "sweep-out" can be avoided by using as low a double resonance oscillator amplitude as possible, that is, working at the "threshold", and/or by increasing the distance between the upper and lower plates. The "sweep-out" or ion ejection technique is a legitimate double resonance experiment provided that the sign of the double resonance signal is not of interest and that care is taken in the interpretation of the results obtained.

At a constant magnetic field strength (B') the m/z values and cyclotron frequencies of the reactant ions (R^+) and product ions (P^+) are related in the following manner.

From equation 2.17

$$\omega_1 = \frac{zB'}{m_P} \qquad 2.22$$

$$\omega_2 = \frac{zB'}{m_R} \qquad 2.23$$

Hence,

$$m_R\ \omega_2 = m_P\ \omega_1 \qquad 2.24$$

$$m_R = m_P\ \frac{\omega_1}{\omega_2} \qquad 2.25$$

Thus the mass of the reactant ions (m_R) that gave rise to the product ions of mass m_P can be calculated. Since the composition and molecular weight of the reacting neutral species (N) is known then the molecular weight of the product neutral(s) (F) can be inferred. A typical double resonance spectrum is shown in Figure 2.9.

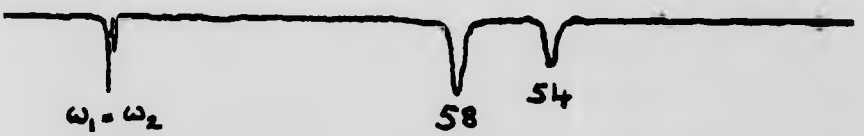


Figure 2.9. Double resonance scan of the ion at *m/z* 80 in the I.C.R. spectrum of vinyl methyl ether and 1,3-butadiene (see Figure 2.7). The double resonance oscillator has an amplitude of 20 V and is swept from 150 to 350 KHz, which corresponds to a mass range of 85 to 35. Double resonance occurs at 220.7 KHz, and 237.0 KHz which corresponds to the *m/z* 58 and 54 respectively (see Section 4.5.1 for an explanation of these signals).

2.4 METASTABLE IONS AND COLLISION INDUCED DECOMPOSITION

2.4.1 Introduction

Ions which undergo a unimolecular decomposition after leaving the ion source of a mass spectrometer and before collection are termed metastable ions. If the product ions of such a decomposition reach the collector then they will be recorded in the mass spectrum as a metastable peak. Due to instrument time scale limitations such unimolecular decompositions must have a rate constant of the order of $10^5\ s^{-1}$.

The origin of metastable peaks was first correctly proposed in 1945[68] by Hipple and Condon. Although metastable ions, like ion-molecule reaction products, were initially considered a nuisance they have since formed the basis of a variety of investigations. Work concerned with ion structures and properties such as kinetic energy release and energy partitioning has been fully discussed by Levsen[69] whereas applications designed to obtain information about neutral compounds such as mixture analysis, the identification of isomers and the interpretation of mass spectra through fragmentation pathways and rearrangements have been reviewed by Schlunegger[70] and Cooks *et al.*[42] Applications of metastable ions pertinent to this thesis involve those of ion structure determination (Chapters 4 and 5) and mixture analysis (Chapter 6). The rest of this section will be devoted to a discussion of the various methods of observing metastable ions together with a discussion of collision induced decomposition.

2.4.2 Methods of Observation

Consider a general metastable transition, equation 2.26 where m_1^+ represents a precursor/parent ion and m_2^+ represents a fragment/daughter ion.

$$m_1^+ \longrightarrow m_2^+ + m \qquad 2.26$$

If such a fragmentation occurs while m_1^+ is under the influence of one of the three variable fields, the accelerating field, the electric sector field or the magnetic sector field, in a double focussing mass spectrometer (Fig. 2.10) then the product ions will almost invariably be lost to the walls of the flight tube.

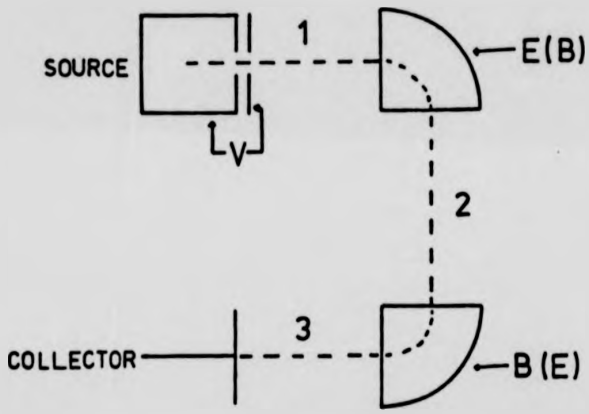


Figure 2.10. Schematic of a double focussing mass spectrometer. (Letters in brackets operative for reverse geometry mass spectrometers.)
V = Accelerating Voltage
E = Electric Sector Field
B = Magnetic Sector Field
1,2,3 = Field Free Regions.

Fragment ions formed in the first or second field free regions, however, can be collected by the appropriate scanning of one or two of the variable fields. With the use of a Daly detector[43] metastable decompositions occurring in the third field free region can be observed. Some of these metastable scans are summarised in Table 2.3.

Table 2.3. Characteristics of Different Metastable Scans

	Scan	Fixed	f.f.r.[a]	Geometry[b]	Peak Shape[c]	Comments	Ref.
(i)	B	V, E	2	N	B	Normal M.S. Metastable $\frac{m_2^2}{m_1}$	71
(ii)	E	V, B	2	R	B	MIKES/DADI Constant m_1	71
(iii)	V	E, B	1	N/R	B	Constant m_2	71
(iv)	$V^{\frac{1}{2}}/E$	B	1	N/R	N	Constant m_1	71
(v)	B/E	V	1	N/R	N	Constant m_1	71/72
(vi)	B^2/E	V	1	N/R	B	Constant m_2	72
(vii)	$\frac{B(1-E)^{\frac{1}{2}}}{E}$	V	1	N/R	N	Constant m	73

(a) f.f.r. = field free region (b) N = Nier Johnson; R = reverse Nier Johnson; N/R = either geometry

(c) B = Broad; N = Narrow.

It should be noted that (i) is not a true metastable scan. It suffers from the disadvantages of weak intensity metastable peaks and overlapping peaks due to the simultaneous collection of ions of different mass formed in the source.

Broadly speaking the scans in Table 2.3 can be split into three groups. The first group, scans (i) and (ii) involve the scanning of one sector only and observe metastable transitions in the second field free region. Hence the geometry of the instrument is important. However, for all scans involving V or linked electric/magnetic sector scans, where first field free region metastable decompositions are being observed, the geometry of the instrument is less important.

The second group of metastable scans (iii) and (iv) involve the scanning of the accelerating voltage (V). Scanning V alters the source focussing conditions and (also) severely limits the mass range over which the scans can be used. Therefore scans (v) and (vi) which give the same information as scans (iv) and (iii) respectively are usually used in preference. The final group of scans (iv), (v) and (vi) all involve the linked scanning of the magnetic (B) and electric (E) fields in various ways and they all give complementary information.

The appropriate choice of metastable scan depends upon the information required. Single sector scans give broad metastable peaks which are good for studying the kinetic energy of the transition but bad for mass assignment of the recorded peak whereas linked scans give rise to narrow peak profiles. Scans (ii), (iv) and (v) give the fragment ions (m_2^+) of a particular precursor ion (m_1^+) whereas scans (iii) and (iv) give the precursor ions (m_1^+) of a particular fragment ion (m_2^+). Scan (vii) is unique in that it allows all the fragment ions (m_2^+) of a particular constant neutral loss to be collected. It can be

seen that if peak shape is not of interest then the three magnetic/electric linked scans which can be performed on an instrument of either geometry will give all the information required. Furthermore if the magnetic sector is repeatedly scanned whilst the electric sector voltage is decremented before each scan then a three dimensional metastable map will be obtained. This map will contain all the information pertinent to scans (v), (vi) and (vii) and will also allow peak shape information to be obtained. Work is currently in progress to construct such a metastable map on a routine basis.[74] The advantages and disadvantages of the above seven scans have been discussed in detail by several authors.[70,71,75,76].

2.4.3 *Collision Induced Decomposition (C.I.D.)*

Some ionic fragmentations occurring outside the ion source of a mass spectrometer are caused by a bimolecular collision between an ion and a neutral molecule, such as a residual gas molecule, followed by a (rapid) unimolecular decomposition. Such collision induced decompositions occur when ions of high translational energy (> 1 KeV) collide inelastically with neutral atoms or molecules. Upon collision part of the translational energy of the ion is converted into electronic excitation energy, thus increasing the internal energy content of the ion. This is called collisional activation (C.A.). The collisionally activated ion may now have the required energy to decompose. Equation 2.27 illustrates this two stage process of fragmentation.

$$m_1^+ + N \longrightarrow \underset{\text{ACTIVATION}}{m_1^{*+}} + N \longrightarrow \underset{\text{DECOMPOSITION}}{m_2^+} + m + N \qquad 2.27$$

The positioning of a collision chamber at a focal point in a field free region of a mass spectrometer facilitates the occurrence of collision induced decompositions. Such decompositions can then be

observed in the same ways as previously described for metastable transitions. The ionic decomposition products of an ion which are formed after a collision constitute a collision induced decomposition spectrum or as it is often less correctly referred to a collisional activation spectrum of that ion.

A study of the variation of the intensity ratios of collision induced fragments as a function of various parameters[77] has revealed the following. The translational energy of the ion not only increases the yield of fragment ions as the energy increases but also alters the intensity ratios. The intensity of fragments having higher activation energies increases more than that of fragments with lower activation energies. A similar increase in the occurrence of high activation energy processes is found at high pressures of collision gas; this is probably due to multiple collisions. The nature of the collision gas has no effect upon the intensity ratios although the yield of fragment ions produced increases as the mass of the collision gas decreases. Commonly used collision gases include hydrogen, helium, nitrogen and argon. Finally, unlike metastable decompositions,[78] the intensity ratios of collision induced fragment ions depends only slightly upon the internal energy distribution of the precursor ion prior to collision.[77,79] Thus a C.I.D. or C.A. spectrum is usually characterised by the percentage decrease in transmission of the precursor ion beam caused by the introduction of the collision gas and the translational energy of the precursor ion beam. For example 50% transmission and 8 KeV translational energy, are typical.

It should be noted that the collision induced decomposition spectra of molecular ions and electron impact spectra of the same molecules show the same types of fragments[77,80] and that the relative fragment intensities in the 70 eV electron impact spectra are very similar to those obtained in the C.I.D. spectra of the same molecular

ions using 8-10 KeV ions.[77,80] This makes it probable that the internal energy distribution of the molecular ions is similar in both cases. However, the similarity in the spectra will also be due to a comparable decomposition time, which is around 10^{-5}-10^{-6} s due to instrumental limitations. The arrangements of the slits in the mass spectrometer means that in order to observe the decomposition products there must be no change or little change in the direction of the primary ion beam and hence the fraction of translational energy transferred to these such ions will be small. The amount of excitation energy deposited in an ion can be estimated by measuring the loss in the translational energy of the ion. This loss in translational energy leads to a shift in the centre of the peak to lower energies which can be measured.[81]

Care must be taken to ensure whether a fragment ion is the product of a metastable decomposition, a collision induced decomposition or both. Metastable decompositions can be characterised by the lack of dependence of the intensity of the metastable peaks upon the pressure of the collision gas. C.I.D./C.A. spectra are independent of the internal energy of the precursor ion for all except the lowest activation energy processes, provided that any metastable processes have been excluded from the spectra.

2.4.4 Constant Precursor Ion Scans

The B/E linked scan, the only metastable linked scan used in this work, is shown schematically in Figure 2.11. Ions m_1^+ of velocity v_1 will be transmitted and collected if the relationships between the kinetic energy, the momentum of the ion, the electric sector field strength (E_o) and the magnetic field strength (B_o) are as follows:

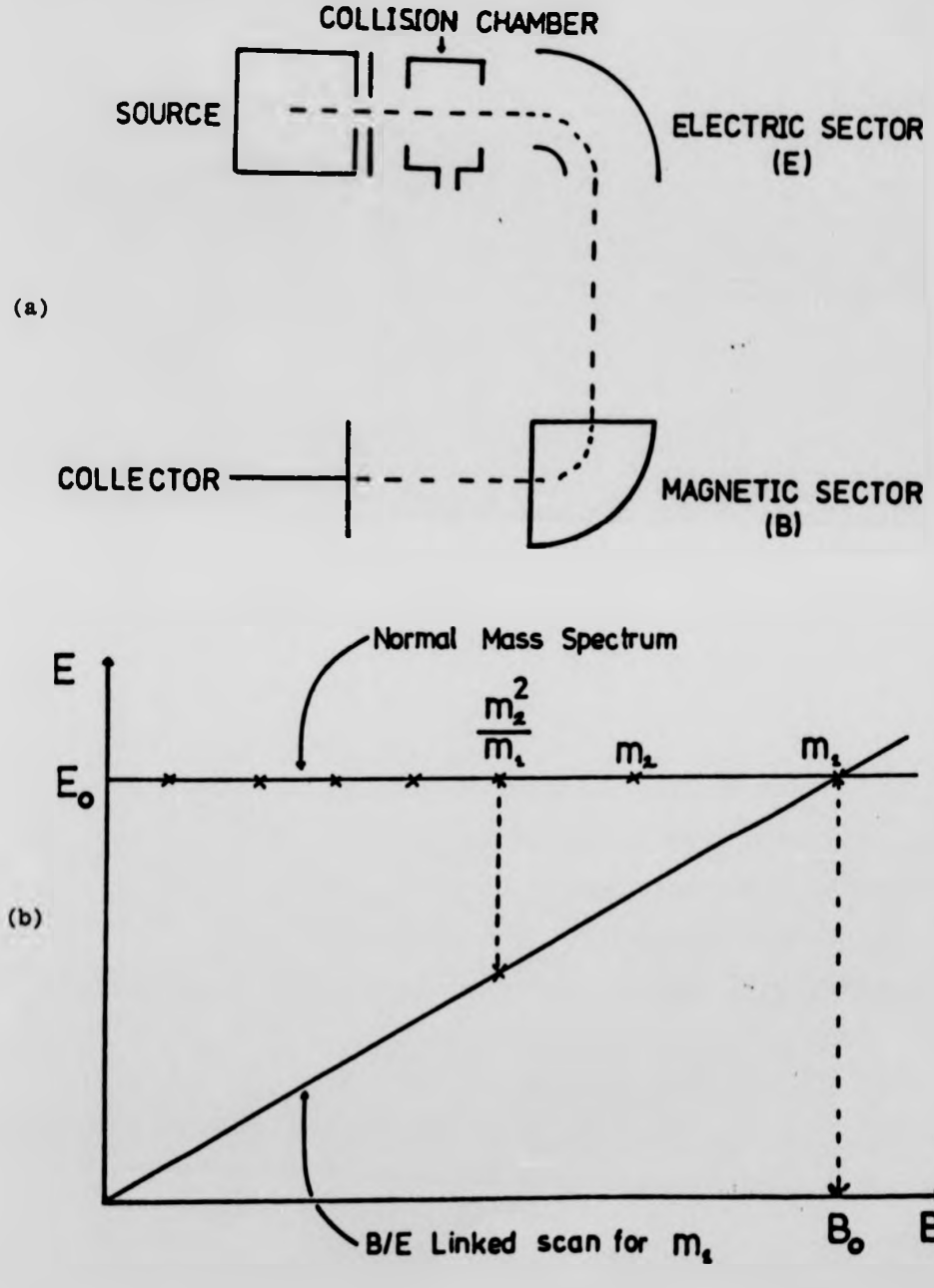


Figure 2.11. B/E Linked Scan.
(a) Instrument schematic.
(b) Representation in terms of B v E plane of a three dimensional mass spectrum.

$$m_1 v_1^2 = eE_o r_e \qquad 2.28$$

$$m_1 v_1 = eB_o r_b \qquad 2.29$$

(where r_e = radius of electric sector

r_b = radius of magnetic sector)

Ions m_2^+ of velocity v_1 formed in reaction $m_1^+ \rightarrow m_2^+ + m$ in the first field free region will be observed if:

$$m_2 v_1^2 = eE_1 r_e \qquad 2.30$$

$$m_2 v_1 = eB_1 r_b \qquad 2.31$$

From these equations it follows that:

$$E_1 = (m_2/m_1)\, E_o \qquad 2.32$$

$$B_1 = (m_2/m_1)\, B_o \qquad 2.33$$

Thus both E and B must be reduced by the same fraction (m_2/m_1) of their original value in order to collect the fragment ion m_2^+. Hence all the fragment ions generated from m_1^+ in the first field free region may be collected if the ratio of B/E is reduced in a constant manner where the constant is determined by the ratio of B/E required to transmit the precursor ions m_1^+ (Figure 2.11(b)). Enhanced fragmentation of m_1^+ will be obtained if a collision cell is placed at the focal point in the first field free region.

An alternative technique to that of B/E linked scanning is provided by mass analysed ion kinetic energy spectrometry (M.I.K.E.S.) Figure 2.12.

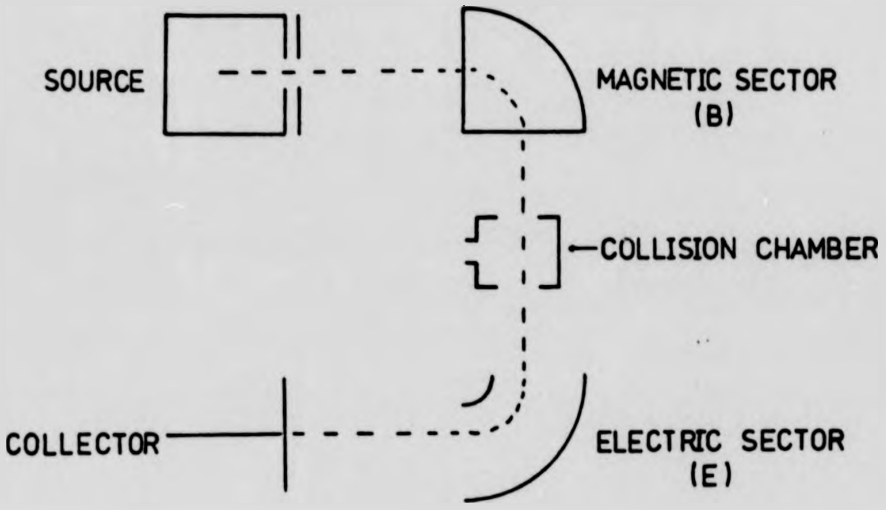


Figure 2.12. Schematic of the instrumentation used for a M.I.K.E.S. experiment.

In this technique, which gives essentially the same information as a B/E linked scan, the magnet is used to select a mass analysed ion beam (m_1^+). The metastable fragment ions (m_2^+) formed in the second field free region from decompositions of m_1^+ are then collected by scanning the electric sector. Hence an ion kinetic energy spectrum of a previously mass analysed ion beam is obtained. Here again enhanced fragmentation of m_1^+ will occur if a collision cell is placed in the second field free region.

Recently a new instrument, the triple quadrupole,[82] has been developed in order to make use of collision induced decompositions for mixture analysis, amongst other things. This mass spectrometer consists of three quadrupole filters, the first produces a mass selected ion beam, the second acts as a collision chamber in which no mass selection occurs and the third is used to monitor the fragment ions produced.

CHAPTER 3

THE LOCATION OF OLEFINIC BONDS

3.1 INTRODUCTION

Small changes in the structure and/or stereochemistry of a compound can often make a large difference to its biological activity.[83] With the increased realisation of the role of such specificity it becomes essential to develop quick, reliable analytical techniques for the elucidation of such structural features.

The structural feature considered here is that of an olefinic or carbon-carbon double bond. Traditionally the positions of such bonds in organic molecules have been determined by a degradation procedure involving the oxidative cleavage of the olefinic bond and subsequent analysis of the products.[84,85] This technique is laborious, time consuming (5 days) and subject to the potential losses of volatile material.[85] In order to overcome these problems various techniques utilising modern analytical instruments have been developed.

In the 1960's two different approaches to olefinic bond location were pursued. The first approach involved the use of a micro-ozoniser coupled to a gas chromatograph(G.C.).[85,86] This method which is essentially a small scale version of the original synthetic route greatly increased both the speed (5 days → 1 hour) and the sensitivity (10-100 μg → 1-5 μg) of analysis. The second approach followed by several workers,[87,88] involved the use of mass spectrometry as this could also provide increased speed and sensitivity of analysis and, if coupled to a gas chromatograph, allow mixtures to be analysed. More recently Nuclear Magnetic Resonance (N.M.R.)[89] has been used to locate olefinic bonds. Although this technique does not have the same sensitivity as mass spectrometry and cannot be applied to mixtures it does allow both the position and the geometry [(*cis/trans* isomerism)] of the olefinic bond to be determined. Indeed

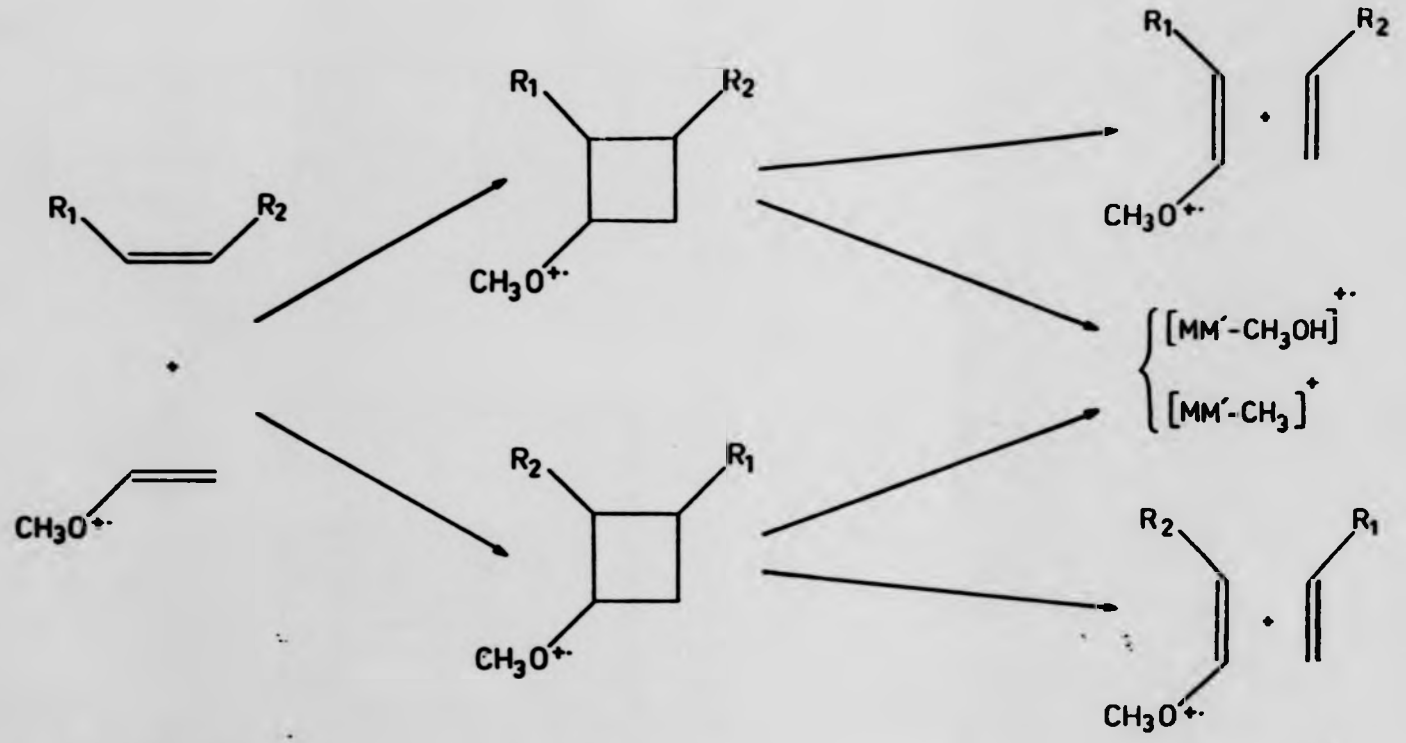
joint application of the techniques of N.M.R. and mass spectrometry have been used to provide a full stereochemical characterisation of a juvenile hormone (ecdysone)[90] and a variety of aliphatic alicyclic compounds.[91]

3.2 MASS SPECTROMETRIC METHODS OF OLEFINIC BOND LOCATION

Under electron impact conditions, olefinic molecular ions invariably isomerise to a common structure or mixture of structures and hence positional information is lost.[92] It has been shown using field ionisation kinetics coupled with deuterium and carbon-13 labelling that linear octene molecular cations isomerise within 10^{-9} s of formation due to hydrogen and radical site migrations.[93] Indeed because of the short source residence time, field ionisation can be used for the location of carbon-carbon double bonds[94,95] but this technique has been applied only to linear alkenes and small carbon branched alkenes so far.

Because of this isomerisation problem and the lack of alternative ionisation techniques such as field ionisation early methods of olefinic bond location were based on the investigation of the mass spectral behaviour of chemical derivatives of the original compounds.[96] Such derivatisation procedures allowed the position of the carbon-carbon double bond in the molecule to be marked by attaching a variety of chemical groups to the olefinic bond. However, these methods were not without their problems, e.g. increased analysis time, lack of sensitivity (100 μg required for derivatisation) and, the reliance in certain cases on low intensity peaks in the mass spectra and the need in some cases for high resolution.

The advent of chemical ionisation led to a new philosophy for the determination of olefinic bond position. The first chemical



(46)

Figure 3.1. General pattern of ion-molecule reactions in the V.M.E./olefin system.

ionisation study[97] using alkenes showed that the use of a proton transfer reaction to produce a protonated molecular ion which subsequently fragmented still gave no positional information about the olefinic bond. A better approach however is provided by an analogous route to the original degradation procedure whereby a reagent gas is used to produce an ion that reacts specifically with the olefinic molecule to give products which vary with the position of the double bond.

Fluoroethylenes,[98] fluoropropenes,[99] isobutane,[100] nitric oxide[100] and an ozone/argon/water[101] mixture are examples of such reagent gases. However, the most general reagent gas for the location of olefinic bonds is vinyl methyl ether (V.M.E.).[102]

3.3 THE VINYL METHYL ETHER* (V.M.E.) TECHNIQUE

*(Vinyl methyl ether is more properly known as 1-methoxyethene)

The general basis of this technique has been described[103-105] previously and Figure 3.1 shows schematically the ion-molecule reactions that are commonly observed using V.M.E. as the reagent gas. A V.M.E. molecule ion reacts with an olefin molecule to give a short-lived complex. This complex then dissociates by one of several competing pathways, one of which (olefin elimination) gives rise to ionic products which are indicative of the position of the olefinic bond in the original molecule. Ions commonly observed from the alternative pathways are those which arise from the loss of a methyl radical or methanol molecule from the complex.

The main disadvantage of the technique initially was that V.M.E. formed positively charged dimers, proton bound dimers and higher polymeric ions which dissociated to give a range of fragment ions.

These reactions both reduce the sensitivity of the method by removing V.M.E. molecule ions and mask peaks of possible interest at the lower end of the mass spectrum. Methods of minimising the occurrence of these reactions were therefore sought.

Initially,[103] a mixture of carbon dioxide and V.M.E. in the ratio 90:10 was used in an attempt to produce V.M.E. molecular ions by charge exchange from carbon dioxide molecular ions. Although this effected a slight improvement, better results might be expected from the use of a charge exchange reagent with an ionisation energy less than that of carbon dioxide (13.79 eV) and only a little higher than that of V.M.E. (8.93 eV). Carbon disulphide fits this requirement and has been used previously in conjunction with nitrogen as a charge exchange reagent.[106] Thus a three component mixture nitrogen/carbon disulphide/V.M.E., where the carbon disulphide acts as a charge exchange reagent to produce low internal energy V.M.E. molecular ions and the nitrogen acts as a buffer gas and diluent, has been used to locate olefinic bonds.

3.4 EXPERIMENTAL

The work was carried out using an MS1073 (Kratos Scientific Instruments Limited) double focussing mass spectrometer equipped with a dual purpose electron impact/chemical ionisation source operating at 1 kV. An ion exit slit of width 0.14 mm and length 3 mm enabled the pressure in the ion source to be maintained sufficiently high to obtain good chemical ionisation conditions. The source pressure was not measured directly but reproducible spectra were obtained by the use of an ion gauge situated above the ion source. A source temperature of 200°C and an electron beam energy of 200 eV were used.

Liquid samples (olefins) were injected through a septum into a

heated reservoir probe and the pressure of the samples within the ion source was controlled by means of a manual leak valve. The reagent gas mixture was prepared in a 2 dm^3 bulb on a gas handling line by subjecting a mixture of carbon disulphide and V.M.E. to several freeze/pump/thaw cycles before the pressure was made up to one atmosphere by the addition of nitrogen. The gas was admitted to the ion source by means of a manual micrometering valve (Whitney) and glass re-entrant tube.

V.M.E. and nitrogen were obtained from Air Products and British Oxygen Company respectively and all other compounds were commercial samples from standard sources.

3.5 RESULTS AND DISCUSSION

3.5.1 Reagent Gas

Initial experiments established that a mixture of composition 75/20/5 (nitrogen/carbon disulphide/V.M.E.) gave optimum results in that it gave an abundant supply of V.M.E. molecular ions without any complications due to polymerisation reactions. The two major ions in a typical reagent ion spectrum are the V.M.E. molecular ion at *m/z* 58 (~ 60% of the total ionisation) and the carbon disulphide molecular ion at *m/z* 76 (~ 20% of the total ionisation). The remaining ions comprise less than 20% of the total ionisation. See Figure 3.2.

3.5.2 Mono-octenes

In each case the spectra obtained with the new reagent gas mixture were simpler than those obtained using V.M.E. alone so that the peaks of interest were observed more readily.

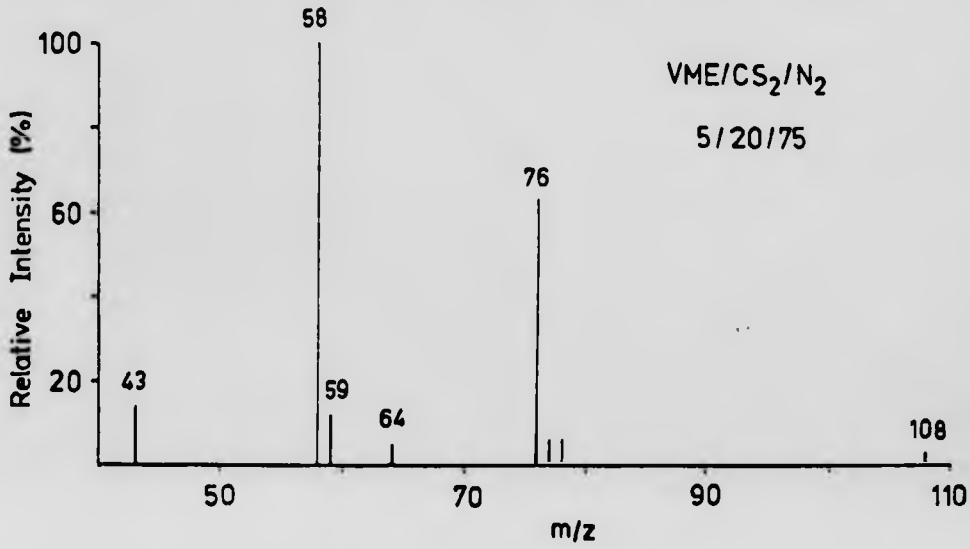


Figure 3.2. V.M.E./CS_2/N_2 reagent ion spectrum.

Figure 3.3 shows a full mass spectrum obtained using the reagent mix and 1-octene, the peak at *m/z* 142 being sufficient to determine the position of the olefinic bond. In the case of terminal olefins such as 1-octene either ethene or the original olefin can be eliminated from the reaction complex. The latter reaction may simply be the back reaction or may involve the elimination of an olefin containing the methylene group originally present in the vinyl methyl ether ion. Hence 1-octene provides no evidence of the formation of two reaction complexes.

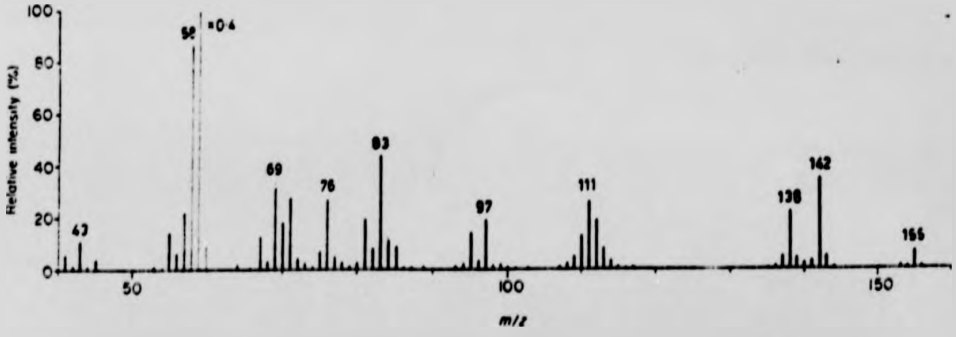
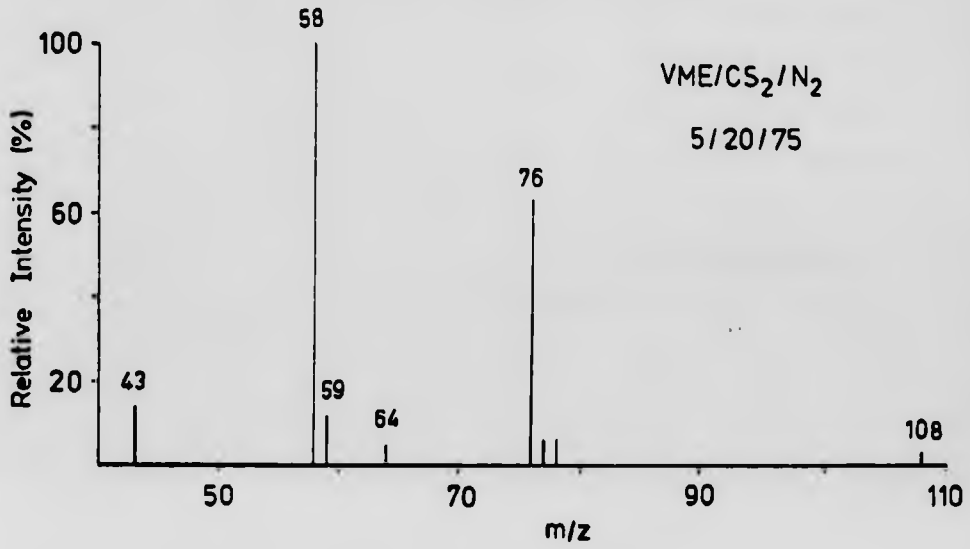


Figure 3.3. Mass spectrum of the olefin 1-octene with the reagent mixture N_2/CS_2/V.M.E.



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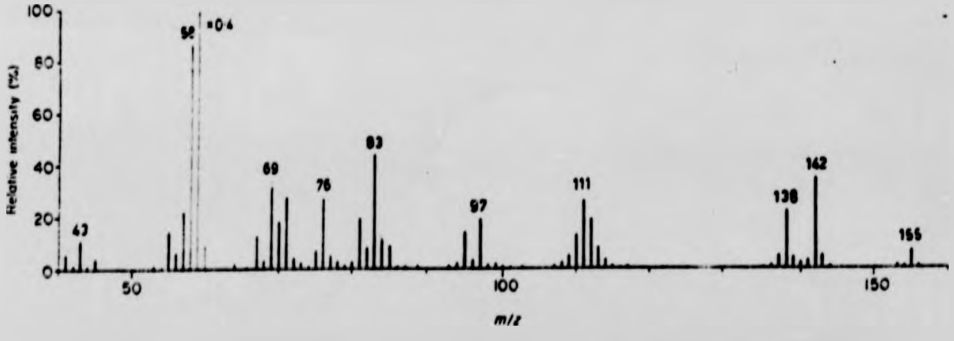


Figure 3.3. Mass spectrum of the olefin 1-octene with the reagent mixture N_2/CS_2/V.M.E.

The relative simplicity of the lower mass end of the spectrum proved useful in the cases of 2-octene and 3-octene. Ions at m/z 72 and 128 (Fig. 3.4(a)) and m/z 86 and 114 (Fig. 3.4 (b)) respectively show that contrary to previous observations[103] two collision complexes are formed, yielding two possible olefinic ion products upon fragmentation (see Figure 3.1). In all the cases we have studied, the charge remains with the olefin containing the methoxyl group.

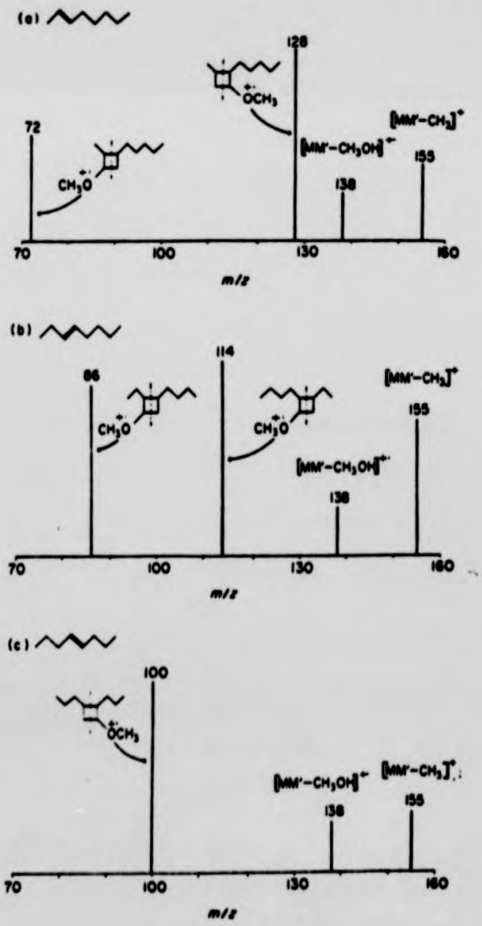


Figure 3.4. Mass spectra of the olefins (a) 2-octene, (b) 3-octene, (c) 4-octene, with the reagent mixture N_2/CS_2/V.M.E.. (The peaks reproduced here are only those arising from reactions between the olefin and V.M.E.)

In the reaction with 2-octene, the two possible collision complexes can eliminate either $C_5H_{11}\cdot CH{=}CH_2$ or $CH_3\cdot CH{=}CH_2$, and as can be seen from the ratio of the relative intensities of the ions at *m/z* 72 and 128 (1:2) the elimination of the smaller olefin $CH_3\cdot CH{=}CH_2$ is more favoured. Either $C_4H_9\cdot CH{=}CH_2$ or $C_2H_5\cdot CH{=}CH_2$ can be eliminated from the two 3-octene/V.M.E. complexes and the ratio of the intensities of the peaks at *m/z* 86 and 114 (5:6) shows that the elimination of the smaller olefin is again favoured. As the two possible neutral olefins being eliminated become similar in size, the ratio of the intensities of the peaks due to the ions formed by their elimination approaches unity.

Due to their symmetry, olefins such as 4-octene (Fig. 3.4(c)) give rise to only one possible collision complex and hence give only one possible olefinic product ion.

3.5.3 Octadienes

The olefin elimination reaction was not observed with the conjugated 1,3-octadiene; the only product observed was that due to the loss of methanol from the reaction complex, consistent with previous results.[107]

The terminal, symmetric, unconjugated 1,7-octadiene (Fig. 3.5(a)) gave a peak at *m/z* 140 corresponding to an ion formed by the elimination of ethene from a collision complex formed with either carbon-carbon double bond. The observation of this peak, together with the absence of all others which would have been expected had one of the double bonds been in a different position, is sufficient to locate the positions of both olefinic bonds.

The symmetric, unconjugated 2,6-octadiene (Fig. 3.5(b)) gave ions of *m/z* 126 and *m/z* 72 which enable one to determine the positions of

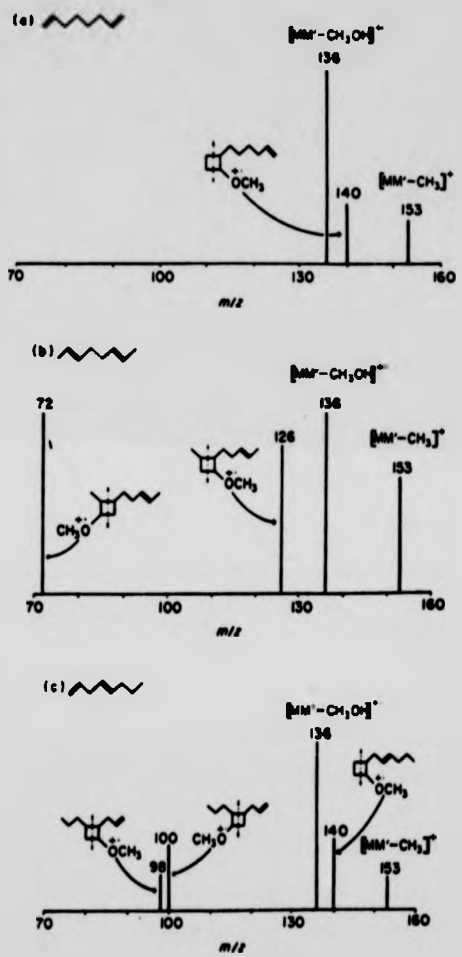


Figure 3.5. Mass spectra of the olefins (a) 1,7-octadiene, (b) 2,6-octadiene, (c) 1,4-octadiene, with the reagent mixture N_2/CS_2/V.M.E.. (The peaks reproduced here are only those arising from reactions between the olefin and V.M.E.)

both double bonds from these observations. On the basis of previous work these ions are assigned the structures $[CH_3 \cdot CH{=}CH \cdot (CH_2)_2 CH{=}CH \cdot OCH_3]^{+\cdot}$ and $[CH_3 \cdot CH{=}CH \cdot OCH_3]^{+\cdot}$ and arise from the fragmentations of the two possible reaction complexes.

The unconjugated 1,4-octadiene gave a more complicated spectrum (Fig. 3.5(c)) due to its lack of symmetry. Nevertheless the positions

of both olefinic bonds can be inferred from the presence of ions of *m/z* 140, 100 and 98. The ions of *m/z* 140 are formed by the elimination of ethene ($CH_2{=}CH_2$) from a reaction complex formed with the terminal double bond. The ions of *m/z* 100 and 98 are assigned the structures $[CH_3{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}OCH_3]^{+\cdot}$ and $[CH_2{=}CH{\cdot}CH_2{\cdot}CH{=}CH{\cdot}OCH_3]^{+\cdot}$ respectively, and are formed by the elimination of an olefin from the two possible reaction complexes formed with the double bond in the 4-position.

Because of the lower ionization energy of dienes and the increased number of fragmentation pathways and possible reaction complexes, the intensities of the peaks due to products of ion-molecule reactions observed in the case of dienes are less than those observed in mono-olefin systems. Nevertheless, the peaks are sufficiently intense for one to be able to assign the positions of the two double bonds unequivocally from a single mass spectrum, thereby extending the utility of the method.

3.6 SCOPE AND LIMITATIONS OF THE V.M.E. TECHNIQUE

The scope and limitations of this technique can be most easily assessed by considering the sensitivity, ease and speed of analysis together with the range of compounds that can be analysed by this method.

No absolute measure of sensitivity has been made for the technique, as obviously this would in part reflect the sensitivity of the mass spectrometer being used. Therefore it should be noted that when using the CS_2/V.M.E./N_2 reagent gas mixture results were obtained routinely using 1 μg of olefinic material. Thus in terms of sensitivity the technique compares favourably with other mass spectrometric techniques and along with field ionisation provides the most sensitive technique for carbon-carbon double bond location. The speed of obtaining a

spectrum is the same as obtaining a normal C.I. spectrum and the analysis of this spectrum is quick because one is looking for the presence of intense peaks of even mass and the absence of intense peaks every fourteen mass units either way from the reaction product peaks. Molecular weight information can be obtained from the V.M.E. C.I. spectrum or else alternate E.I./C.I. spectra can be obtained. By using this latter technique, one can subtract the electron impact spectrum from the V.M.E. C.I. spectrum to leave the reaction product ions. Mixtures can be analysed using G.C./M.S. although this may place some requirement on derivatisation to stop decomposition on the G.C. column. Instrumental demands for the technique are not high, the only specialised requirement being for chemical ionisation. The main experimental restriction is the (5/20/75) V.M.E./CS_2/N_2 mixture; however, these substances are all easily obtainable and the mixture can readily be made up, using a simple vacuum line, and stored in a large glass bulb.

Several papers have now been published[102-105,108,109] using the V.M.E. technique for a variety of compounds. In order to establish more about the range of compounds for which this technique is applicable experiments were performed on selected compounds in order that these results together with those already published would cover as many different types of olefinic compounds as possible. For the sake of clarity all compounds containing olefinic bonds have been broken down into eight classes as shown in Figure 3.6.

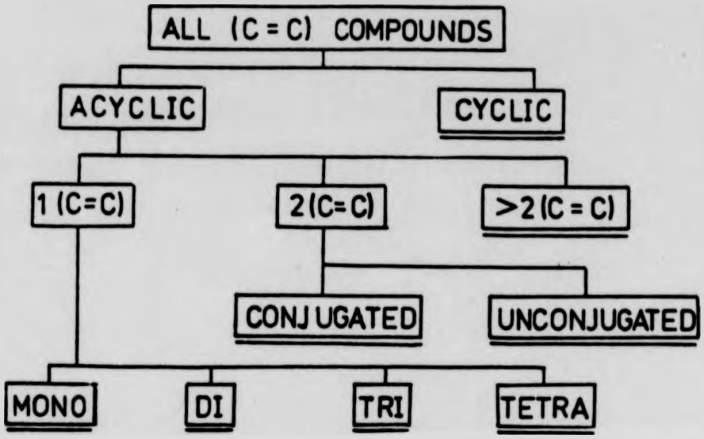


Figure 3.6. Classification of (C=C) containing compounds.

Cyclic Compounds

Cyclic compounds will not undergo the olefin elimination reaction as this would necessitate the breaking of an additional bond in the ring. [Addition of V.M.E.$^{+\cdot}$ with subsequent elimination of methanol can be observed in certain cases.]

> 2(C=C)

The position of olefinic bonds in compounds containing more than two double bonds cannot be found by this technique.

Conjugated Dienes

Conjugated dienes have been shown to undergo a Diels Alder reaction with the V.M.E. molecular ion to form a complex which can subsequently lose methanol. Hence V.M.E. can only be used to indicate the possible presence and not the position of conjugated olefinic bonds in dienes.

Unconjugated Dienes

As shown previously, V.M.E. can be used to locate both the double bonds in unconjugated unsubstituted linear dienes. Because of the increased number of different reaction product ions and because of the lower ionisation energies the technique is less sensitive for dienes than for mono-olefins. So far no success has been achieved for the location of olefinic bonds in substituted unconjugated dienes.

1(C=C)

Single olefinic bond containing compounds have been divided into four classes according to the degree of substitution at the double bond. These four classes will be considered in groups of two.

Tetra- and Tri-substituted (C=C)

Tetra-substituted olefinic bonds, for example, 2,3-dimethyl-2-butene (2,3-dimethyl-2-pentene) and tri-substituted olefinic bonds, for example, 3-methyl-2-buten-1-ol, 2-bromo-2-butene, 2-methyl-2-heptene, and 2,4-dimethyl-2-pentene give no peaks indicative of the double bond position or the formation of a complex. This is most likely due to steric hinderence.

Mono- and Di-substituted (C=C)

It should be noted that mono-substituted olefinic bonds must always be terminal olefins whereas di-substituted olefinic bonds can either be terminal olefins or else they can be substituted so as to produce an internal olefinic bond with *cis* or *trans* configuration. See Figure 3.7.

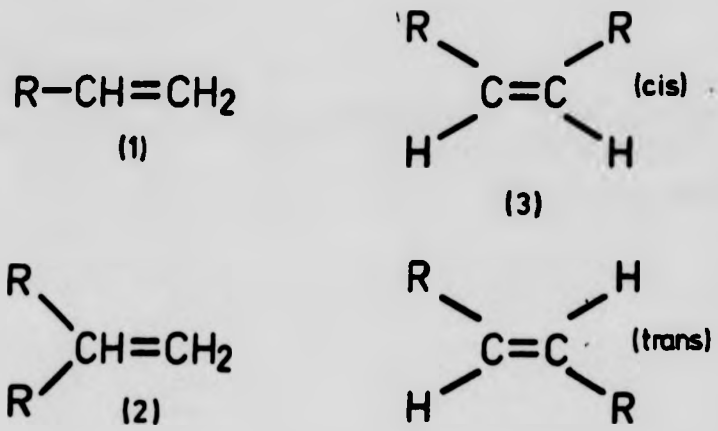
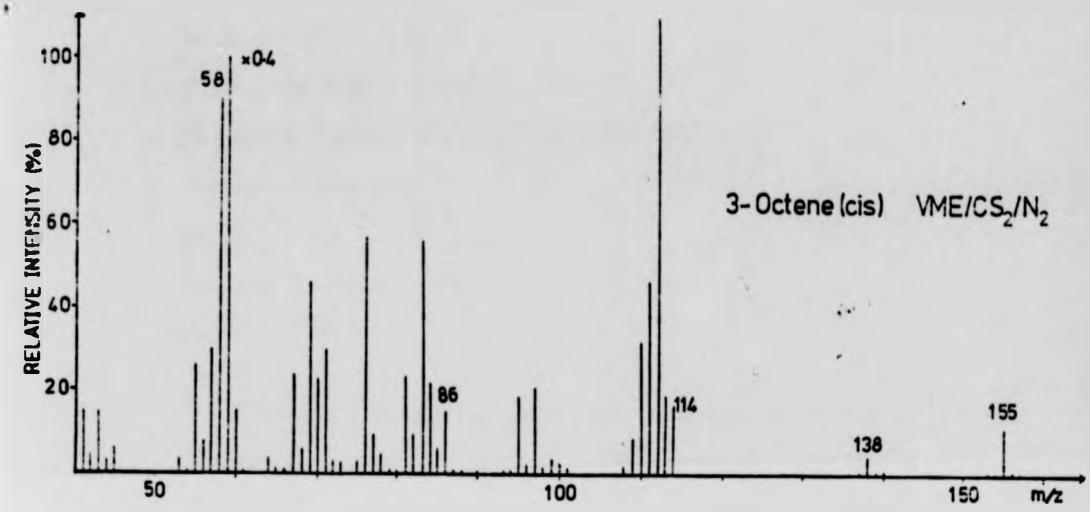


Figure 3.7. Stereochemistry of mono- and di-substituted olefins.
(1) = Mono-substituted terminal olefin.
(2) = Di-substituted terminal olefin.
(3) = Di-substituted internal olefin (*cis/trans*).

The intensity of the ions characteristic of the olefinic bond position are lower for internal olefins (3) than for mono-substituted olefins (1). Compare Oct-3-ene (Figure 3.7) with Oct-1-ene (Figure 3.3.). Alkyl substitution to give a terminal di-substituted olefin (2), for example 2-methyl-1-pentene, also lowers the intensity of such product ions compared with those given by mono-substituted olefins (1); however as the alkyl substituent is moved further away from the double bond their intensity increases, for example methyl-1-hexenes.[108]

A similar trend is observed for non-alkyl substituents in that the nearer such substituents are to the double bond, the more they affect the reaction. Substitution of a functional group directly on to an olefinic bond, for example acrylonitrile, crotonitrile, crotonaldehyde, fluoroethylenes and vinyl chloride prevents the vinyl methyl ether ion from reacting with the olefinic bond. One exception to this is vinyl ethyl ether. Functional groups that are one or two carbon atoms away from a mono substituted olefin do not in general restrict the reaction, for



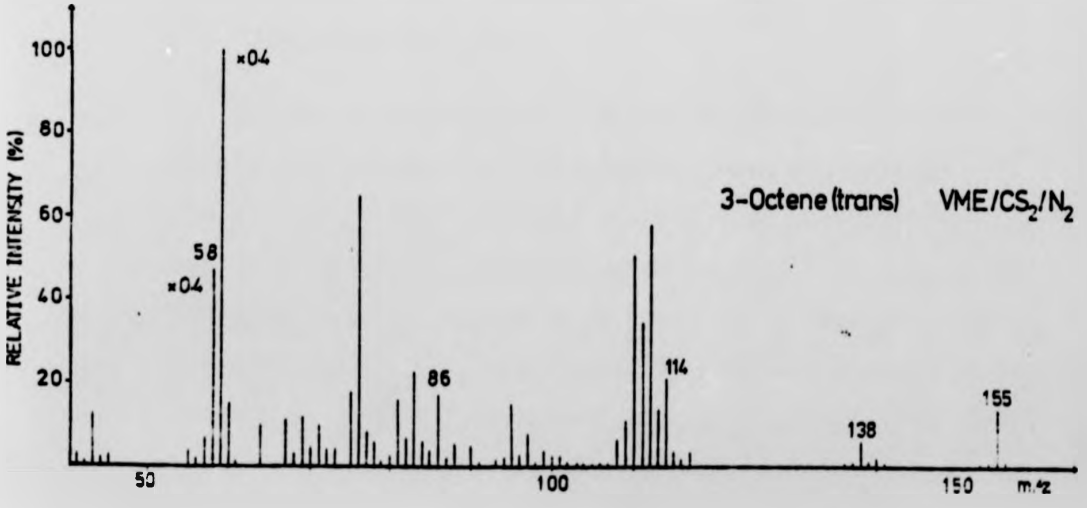


Figure 3.8. Spectra of *cis*-and *trans*-Oct-3-ene with V.M.E./CS_2/N_2.

example butenoic acid, propene acetate and allyl formate. Exceptions to this, where the reaction did not occur, contain electronegative groups, for example 4-hydroxy-1-pentene and 3-fluoro-propene. If the functional group is well removed from the olefinic bond then the technique works well, for example undecenyl alcohol, oleic acid. Indeed the V.M.E. technique has been shown to be useful for the location of olefinic bonds in a number of pheromones (C_{10} methyl acetates).[109]

Finally it should be noted that in general *trans*-isomers appear to react to a greater extent than *cis*-isomers, see Figure 3.8. However, this difference is so small that the geometry of the olefinic bond can be ascertained only by running pure samples of the two isomers.

3.7 CONCLUDING REMARKS

Alternative methods currently in use for the location of olefinic bonds involve the use of C.I. to transfer a proton to a derivatised olefin[110] and the use of isobutane or nitric oxide to produce reagent ions which cleave the olefinic bond in the gas phase.[100] In both cases information about the position of the double bond is obtained from the fragment ions collected. The derivatisation method requires 100-200 µg of sample and involves time consuming derivation and analysis of complex spectra, but is applicable to a large range of compounds. The use of isobutane and nitric oxide appears to be limited to fewer types of compounds than the V.M.E. technique, particularly now that the V.M.E. reagent ion spectrum is simpler. For example nitric oxide cannot be used when the carbon chain is less than ten carbon atoms long. Particularly noteworthy is the fact that isobutane allows *trans*-isomers to be detected more readily than *cis*-isomers due to the increased reactivity of the former.

Thus the use of vinyl methyl ether provides one of the quickest, easiest and most sensitive techniques for the location of olefinic bonds. The main restriction on the widespread use of the technique is the range of compounds that can be analysed by this method. The use of a charge exchange reagent, for example benzene (I.E. = 9.25 eV), with a lower ionisation energy (I.E.) than carbon disulphide (I.E. = 10.08 eV) may further improve the sensitivity of the technique by reducing the amount of olefinic ions produced by charge transfer.

Finally a very intense $(M-2)^{+\cdot}$ ion, where M represents the olefin, appears in several spectra examined in both this laboratory (see Figure 3.8 (b)) and elsewhere.[100] Examination of the formation of this ion in an ion cyclotron resonance spectrometer indicates that the V.M.E. molecular ion is the precursor ion. Thus the V.M.E. radical cation appears to convert some of the olefin into a diene molecular ion.

CHAPTER 4

ION-MOLECULE REACTIONS OF SOME $C_3H_6O^{+\cdot}$ ISOMERS

4.1 ION STRUCTURE

4.1.1 Introduction

The elucidation of the structure of a compound by mass spectrometry is dependent upon an understanding of the structure of the ions formed in the mass spectrometer. Thus one of the most important questions concerning the properties of organic ions is that of structure determination. Olah and his coworkers[111] have made an extensive study of the structure of ions in solution. Whereas the synthesis of ionic species in solution is not easy the corresponding gaseous ions can be formed readily as isolated species in a mass spectrometer. Furthermore the results of ionic structure studies in the gas phase are free from solvation effects and are thus more amenable to comparison with the results of molecular orbital calculations.

Early mass spectrometric methods of ion structure determination relied upon the elucidation of the decomposition mechanisms of excited organic ions using primarily chemical intuition, supported by metastable isotopic labelling studies. Consider three of the decompositions of the 2-hexanone molecular ion shown in Figure 4.1.

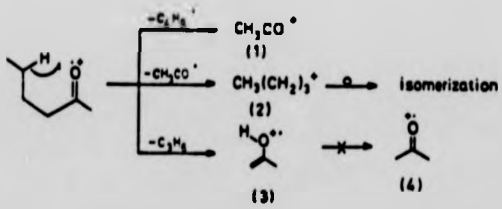


Figure 4.1. Schematic of three major fragmentations of the 2-hexanone molecular ion.

Simple examination of the mass spectrum, coupled with deuterium labelling in the case of the formation of ion structure (3), suggests that ions of

structure (1), (2) and (3) are formed.[112] Additional experiments however have shown that excited n-butyl ions isomerise to a mixture of interconverting structures[113] whereas the enolic ion (3) does not isomerise to the ketonic form (4).[114] Thus a knowledge of the decomposition mechanism gives information about the initial structures of the fragment ions not about the final ionic structures. Furthermore by this method isotopic labelling can only be used to infer which atom is transferred and not where to. Isotope effects, isotopic scrambling and competing decompositions giving various structures of identical composition may also complicate the situation. Several methods have been developed to try and overcome these problems. Such methods together with a more detailed description of the elucidation of reaction mechanisms have been discussed comprehensively by Levsen.[115]

4.1.2 *Definitions*

Methods for the determination of ion structure fall into two groups. The experimental methods give mainly information concerning the constitution of an ion whereas the spectroscopic and theoretical methods provide information about both the constitution and configuration (bond angles and lengths) of an ion. Only ion constitution will be considered in this work.

As seen in the previous example it is necessary to differentiate between the initial structure of an ion when it has just been formed either by ionisation or fragmentation and the final structure of an ion when it is observed. Most ion structure techniques provide information on the final structure of the ions with lifetimes (t) in the range $10^{-6} \leq t \leq 10^{-3}$ s but some methods, for example field ionisation kinetics where t can be as small as 10^{-11} s allow conclusions to be drawn about initial structures.

Ion structure is dependent upon the internal energy of the ion hence it is necessary to differentiate between decomposing ions, those with enough energy to decompose, non-decomposing ions, those which do not decompose within the time scale of the experiment but which may isomerise and stable ions, those which do not decompose or have enough energy to isomerise. These definitions are represented schematically in Figure 4.2.

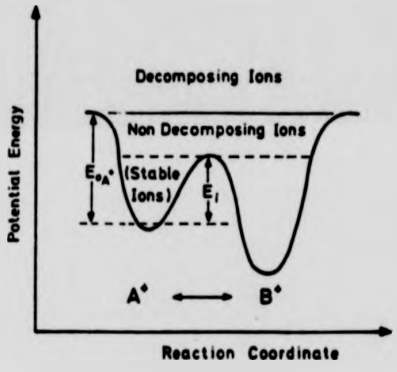


Figure 4.2. Schematic representation of the definitions of decomposing, non-decomposing and stable ions (Adapted from Ref. 34, page 211).

These definitions are rather arbitrary and will be discussed further in terms of isomerisation. In general, most ions will decompose from their original structure faster than they isomerise as witnessed by the widespread use of mass spectrometry for neutral structure determination.

4.1.3 Isomerisation

After electron impact ionisation the structure of the molecular ion (M^+) formed will be known with certainty for 10^{-14} s. After this time interval the ion may decompose either directly or after an intramolecular rearrangement, the controlling factor being a kinetic

one only. That is, the fastest reaction for the molecular ions ($M^{+\cdot}$) of a particular energy will be that which has the greatest rate constant at that energy. Thus a molecular ion may rearrange only if its internal energy ($E_{M^{+\cdot}}$) is greater than or equal to the energy of activation for isomerisation (E_I). Whether this rearrangement actually occurs will then depend upon the relative magnitudes of the rate constants for decomposition (k_D) and for isomerisation (k_I). The various possibilities are summarised in Table 4.1.

Table 4.1. Summary of the conditions for isomerisation. (Adapted from Ref. 33, page 203)

	WILL $M^{+\cdot}$ ISOMERISE?	
	Low $E_{M^{+\cdot}}$	High $E_{M^{+\cdot}}$
(1) $E_I > E_D$; $k_D > k_I$	NO	NO
(2) $E_I > E_D$; $k_I > k_D$	NO	YES
(3) $E_D > E_I$; $k_D > k_I$	YES*	NO
(4) $E_D > E_I$ $k_I > k_D$	YES*	YES

* No isomerisation will occur if $E_{M^{+\cdot}} < E_I$

In conclusion, the extent of isomerisation is dependent upon the internal energy content of the ion. Secondly, the existence of ions of one particular ionic structure does not preclude the existence of other ions of isomeric structures in equilibrium with ions of the former structure. Thirdly, in order to restrict isomerisation as much as possible, low internal energy ions must be produced. These conclusions apply equally to molecular ions and fragment ions.

4.2 REVIEW OF METHODS FOR STRUCTURE ELUCIDATION

4.2.1 *Introduction*

With the use of such spectroscopic techniques as infra-red (I.R.), ultra-violet (U.V.), nuclear magnetic resonance (N.M.R.) and X-ray analysis the confirmation of a chemical's structure has become simple in relation to the problems of synthesis. On the other hand, synthesis of a variety of gas phase ionic species and their separation into pure components is relatively simple compared with the determination of their structure. In general, spectroscopic techniques (e.g. I.R., U.V.) cannot be used to characterise an ion beam owing to the low concentration of the ions. Hence most of the methods used for structure elucidation are akin to the earlier methods of organic structure determination in that they rely on degradation and reactivity studies of the ion of interest. Methods which have been employed include:-

(1) Metastable Ion Characteristics (MI)

(2) Kinetic Energy Release (T)

(3) Collisional Activation Spectra (CA)

(4) Ion-Molecule Reactions (ICR)

(5) Isotopic Labelling (L)

(6) Heats of Formation (H)

(7) Electron Impact Studies (EI)

(8) Molecular Orbital Calculations (MO)

(9) Charge Stripping (CS)

(10) Field Ionisation Kinetics (FIK)

(11) Photoion-Photoelectron Coincidence (PIPECO)

(12) Potential Energy Surfaces (PE)

(13) Experimental Determination of Rate Constants

(14) Photodissociation/I.C.R.

(15) Decomposition Pathways.

There are examples in the literature of structural studies of over one hundred different ions, many ions being studied by more than one technique. For example the $C_2H_5O^{+\cdot}$ isomers have been studied by at least seven different techniques in over twenty publications. Ion structure elucidation techniques have been discussed by several authors[116] with the most comprehensive review of techniques and results being by Levsen.[115] Most of the above techniques rely on a comparison of the unknown ion structure with that of a reference ion. Thus if two ions give the same C.A. spectra or exhibit the same ion-molecule reactions the conclusion is made that they have identical structures and vice versa. This can cause problems when reference ions of known structure are not available and assumes that the given technique is sufficiently sensitive to distinguish between different ion structures present simultaneously.

4.2.2 *Metastable Ion Characteristics*

The principle of this method is that if two isomeric ions give rise to not only the same metastable fragment ions but also the same relative abundances of fragment ions the isomeric ions must have identical structures and vice versa. This hypothesis is a consequence of the Q.E.T. whereby the abundance of a fragment ion ($[Ai^+]$) is a function of the internal energy distribution of the decomposing ion (P(E)) and the rate constant for the formation of the fragment ion (ki)[117] as given in the equation 4.1.

$$[Ai^+] = \int_{\epsilon_{oi}}^{\infty} P(E) \frac{ki}{\sum_{i}^{n} ki} (1-\exp(-\sum_{i}^{n} kit))\ dE \qquad 4.1$$

The rate constant (ki) is itself a function of the activation energy for further decomposition, vibrational and rotational frequencies and moments of inertia in the reactant and activated complex. Two isomeric ions will in general differ in at least one of these variables and hence

will differ in ki. Thus the principle of this method will be valid if the internal energy distribution of the two decomposing ions is the same. Because this technique samples decomposing ions with a range of internal energies 0-1 eV above that required for decomposition and with a lifetime of 10^{-5}-10^{-6} s, this assumption is of doubtful validity. Indeed it has been shown that pronounced differences in the internal energy of ions of identical structure may lead to variations in the relative abundance ratios by a factor of two to five.[78]

Therefore the conclusion that similar metastable ion spectra, within this reproducibility range, implies identical ionic structures is more certain than the conclusion that dissimilar metastable ion spectra imply that the ionic structures are different.

Apart from this disadvantage, there are two others, namely, that structure assignment is often based upon only a few peaks and that the observed metastable fragments are often produced via rearrangement reactions which are less specific than direct fragmentations. The advantages of this technique are that no specialised equipment is needed (although a MIKES or B/E linked scan is an advantage), the internal energy and ion lifetimes are well defined and isotopic labelling can be used to confirm any structural assignment. The field ionisation kinetics technique[57] offers a valuable extension of this technique allowing ions of lifetime 10^{-11}-10^{-5} s to be sampled.

4.2.3 *Kinetic Energy Release*

The conversion of excess internal energy into kinetic energy (T) when a metastable ion decomposes leads to a broadening of the metastable peak width owing to the range of translational energy produced in the fragment ion beam. Hence the ion peak width can be used to calculate the kinetic energy release[118] and characterise the ion

structure.[119] with identical kinetic energy release data implying identical structures and vice versa.

As shown in Figure 4.3 the kinetic energy released T (total) has two possible sources, neglecting tunnelling and pre-dissociation,

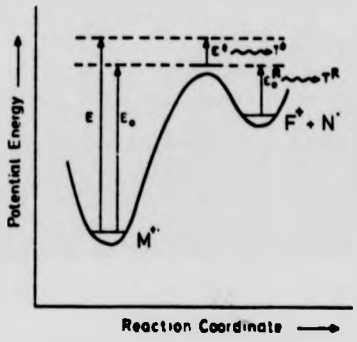


Figure 4.3. Potential energy diagram for the metastable decomposition $M^{+\cdot} \rightarrow F^+ + N^\cdot$ (Adapted from page 134, reference 34).

namely the variable energy above the barrier for activation $\varepsilon^{\ddagger}$ and the reverse activation energy ε_o^R. Thus the total kinetic energy released, as shown in equation 4.2, is given by the contributions

$$T_{TOTAL} = T^{\ddagger} + T^R \qquad 4.2$$

from these two sources. It should be noted that most simple bond cleavages have only small or zero reverse activation barriers whilst rearrangement reactions may exhibit a substantial reverse activation barrier.[120] If two ions have identical structure then their potential energy surfaces and reverse activation energies will be the same and hence T^R will be the same. $T^{\ddagger}$ however will be dependent upon the internal energy of the metastable ions. Thus in the case of ions with a small T^R contribution the kinetic energy release might be expected to show a strong dependence upon the internal energy of the precursor ions. Indeed this has been observed using the photoion-photoelectron

coincidence (PIPECO) technique;[121] as far as metastable ions are concerned however no effect on the metastable peak width has been owing to the variance of internal energy.[118]

This method of ion structure determination samples decomposing ions with internal energy 0-1 eV above threshold and ion lifetimes of 10^{-5}-10^{-6} s. Since the ion structure is determined prior to decomposition the possibility of isomerisation and subsequent decomposition from a different structure, intermediate structure or mixture of structures must be borne in mind. (The possibility that two ions of distinctly different structures may accidentally decompose with the same kinetic energy release cannot be ruled out.) Finally the technique requires the use of double focussing mass spectrometers with good energy resolution and electronic stability in order to obtain reproducible measurements.

Despite the limitations of this method it does constitute one of the most powerful methods of structure determination. It is particularly useful for revealing the presence of ion mixtures and can be used in conjunction with the structure elucidation methods of decomposition mechanisms by isotopic labelling and metastable ion characteristics because all these methods sample decomposing ions with lifetimes of 10^{-6}-10^{-5} s.

4.2.4 Collisional Activation (C.A.)

This method which is similar to that of metastable ion characteristics relies upon the use of the relative abundances of all the collision induced fragment ions of a chosen ion (i.e. C.A. spectrum) to reflect the structure of that ion. Thus identical C.A. spectra imply identical structures and vice versa; the justification for this is the same as for metastable ion characteristics. C.A. methods are

more suited to the role of structure elucidation than M.I. characteristics[122,123] owing to their diminished dependence upon the internal energy of the precursor ion.[122] Indeed the processes of lowest activation energy, indicated by the presence of a strong peak in the metastable ion spectra, are usually excluded from the normalisation of the C.A. spectra for this reason. It is preferable to do this than to try to correct the peak intensity for contributions from unimolecular decompositions. After the exclusion of peaks containing contributions from unimolecular decompositions, if the C.A. spectra vary with electron energy (Excitation Energy) then there must be a mixture of non-interconverting structures present, due to their simultaneous formation from the precursor ion by competing mechanisms with different activation energies. Alternatively, if the C.A. spectra show little variance with electron energy then either a single structure or a mixture of interconverting structures is present. (A mixture of non-interconverting structures with similar energetic and kinetic parameters may also be present.)

This method samples non-decomposing ions with a range of internal energies from zero up to the lowest threshold for decomposition and with a lifetime in the range 10^{-5}-10^{-6} s. It should be noted that after collisional activation, ions may undergo isomerisations that are not accessible to non-decomposing ions. Such highly excited ions will decompose from their original structure at a rate equal to or greater than that for isomerisation. Thus even if the threshold for isomerisation of A^+ to B^+ is below that for decomposition, the C.A. spectra of A^+ and B^+ will be distinct.[124]

In conclusion, the advantages of C.A. are that C.A. leads to a large variety of fragment ions which are more abundant than metastable ions. If processes with unimolecular components are excluded, the C.A. spectrum is largely independent of the internal energy of the precursor ions.

This means that the spectra are reproducible, with relative ion abundances of ≤ 10% variation and small variations (≥ 10%) being structurally significant. If the electron energy is varied, ion mixtures can be detected and with the aid of reference spectra the relative composition of the mixture can be determined semi-quantitatively. C.A. spectra can be obtained in the same way as metastable ion spectra, the only additional requirement being a collision chamber in the appropriate field free region of the mass spectrometer. C.A. spectra can be used in combination with isotopic labelling to give information about structurally significant direct bond cleavages. The kinetic energy released upon C.I.D. can also be used as a criterion for structure elucidation.

The disadvantages of the technique are that the internal energy of the ions is not well defined and if the threshold for isomerisation is much lower than that for decomposition then isomerisation both before and after collisional excitation may obscure differences in the C.A. spectra. Hence two stable ions with a low activation barrier for interconversion may give similar spectra. Thus other techniques which sample non-decomposing ions, e.g. I.C.R., may allow the identification of stable isomers more readily than C.A..

4.2.6 *Ion-Molecule Reactions (I.C.R.)*

The three methods of structure elucidation discussed previously have relied upon the observation of various properties of the degradation of the ion of interest. An alternative philosophy is to use the reactivity of an ion with a neutral molecule as a criterion for structure elucidation. Three different approaches have thus been used. The most usual approach and the one adopted for work reported in this thesis is to compare ion reactivities. Namely, if two isomeric ions undergo the same ion-molecule reaction this implies that they are

structurally identical and vice versa. Secondly, proton transfer reactions to a base, in conjunction with isotopic labelling may be used.[125] Thirdly, the fact that equilibrium constants for ion-molecule reactions of isomeric ions may differ substantially can be used.[125]

Although ion-molecule reactions can be studied by a variety of techniques such as tandem mass spectrometry[13] and chemical ionisation,[50] the technique of ion cyclotron resonance[16,17] is particularly suited to this problem because it allows the product/reactant ion relationship to be established by means of the double resonance technique. Hence the ion-molecule reaction method for structure elucidation has become synonomous with the I.C.R. method.

This method relies upon finding an ion-molecule reaction that is specific for <u>one</u> isomeric form of the ion of interest. The observation or lack of observation of this reaction is then used to demonstrate the presence or absence of one isomeric form. While this is a powerful method for ion structure determination, the instrumentation is not as widely available as that used in mass spectrometry and it is often quite difficult to find suitable ion-molecule reactions to characterise an ionic structure. Therefore care has to be used when interpreting results, particularly those where little is known about the system either from other I.C.R. work or from the use of other techniques. If an ion-molecule reaction has been shown to exist for one particular isomeric form and to be specific for that isomeric structure then it can be used as a reference reaction. The <u>presence</u> of this reaction will then indicate the presence of one isomeric structure. But the <u>absence</u> of the reaction could be due to the ion having a different isomeric structure or mixture of structures or to the fact that the ions formed with the correct structure have the wrong internal energy to react or are present in too low a concentration to be observed.

Indeed it has been pointed out that the occurrence and extent of an ion-molecule reaction may in certain cases depend upon the internal energy of the ions.[126]

Ions of a wide range of internal energies and ion lifetimes are produced in an I.C.R. cell. Those ions with the longest lifetimes ($t \geq 10^{-3}$ s) will have the greatest chance of reacting. The I.C.R. method therefore samples predominantly non-decomposing ions with lifetimes $\geq 10^{-3}$ s. Except for the different lifetime window the results obtained should be comparable with those obtained using collisional activation.

4.2.6 *Other Techniques*

The various other techniques for ion structure elucidation have been reviewed.[115] These techniques will not be discussed here because either they do not constitute a major experimental technique for ion structure determination or they are not relevant to the discussion of the work presented in this thesis.

4.2.7 *Comparison of Techniques*

Before presenting results concerned with two different isomeric groups of ions ($C_3H_6O^{+\cdot}$ and $C_4H_5N^{+\cdot}$) utilising the ion-molecule reaction (I.C.R.) technique, it is important to compare the various techniques of ion structure elucidation in order to establish whether different techniques are expected to give similar results or not.

Owing to the different internal energies and ion lifetimes of the ions sampled by the four techniques given below, identical results should not be expected with each technique. Whether identical results are obtained or not will depend upon the ratio of the activation barriers for isomerisation and decomposition. If no

Table 4.2. Comparison of the internal energy content and lifetime of the ions sampled by various ion structure determination methods. (Adapted from page 217 in reference 34)

TECHNIQUE	INTERNAL ENERGY	ION LIFE-TIMES (s)
M.I.	$\sim E_o^*$	10^{-6}-10^{-5}
T	$\sim E_o^*$	10^{-6}-10^{-5}
C.A.	$0 - E_o$	10^{-6}-10^{-5}
I.C.R.	$0 - E_o$	$\geq 10^{-3}$

E_o = lowest threshold for decomposition.

* = N.B. may be up to 1 eV above E_o.

isomerisation occurs all the techniques will give the same answer, assuming that they are equally capable of distinguishing the ion structures. whereas if isomerisation can occur then the results of the various techniques may differ. Finally it may not be possible to detect very small isomerisation barriers using C.A. and I.C.R. methods and hence the results may represent only a small fraction of the ions present.

Hence if the methods which sample ions with enough energy to decompose (M.I., T) imply that two isomeric ions have distinct structures, all the methods which sample non-decomposing ions (C.A., I.C.R.) must show the same results. The reverse conclusion however cannot be made.

4.1.5 *Concluding Remarks*

In conclusion the main aim of structure elucidation is to gain a knowledge of the structure of an ion under defined conditions. The inability to control finely or to select the internal energy of the ions being studied presents the greatest problem towards achieving this goal

4.3 $C_3H_6O^{+\cdot}$ ISOMERS - INTRODUCTION

4.3.1 Introduction

Since the well known McLafferty rearrangement reaction was first reported in 1959,[112] the $C_3H_6O^{+\cdot}$ system has received considerable attention, making it one of the best understood gas phase ionic systems. The experimental studies concerned with $C_3H_6O^{+\cdot}$ ions have encompassed a variety of techniques and a historical review of these studies serves to illustrate the shortcomings of early metastable ion work and the advantages of ion cyclotron resonance and collisional activation methods for ion structure determination.

The twelve $C_3H_6O^{+\cdot}$ isomeric structures considered in this Chapter are listed in Figure 4.4. Table 4.3 lists the calculated relative energies of these isomers and, where known, their experimentally determined heats of formation.

Table 4.3. Relative energies, heats of formation and neutral precursors for the $C_3H_6O^{+\cdot}$ ions of structure (i)-(xii) shown in Figure 4.4.

Ion Structure	Rel. E.[127] KJ mole^{-1}	ΔH_f KJ mole^{-1}	Test Compounds
i	0	661	Hexan-2-one
ii	14	665	2-methyl Pentanal
iii	20	720	Acetone
iv	53	749	V.M.E.
v	92	770	Propanal
vi	107	-	?
vii	111	-	?
viii	112	-	?
ix	148	-	1,4-Dioxan
x	157	852	Tri-Methylene Oxide
xi	207	893	Propylene Oxide
xii	229	-	Cyclopropanol

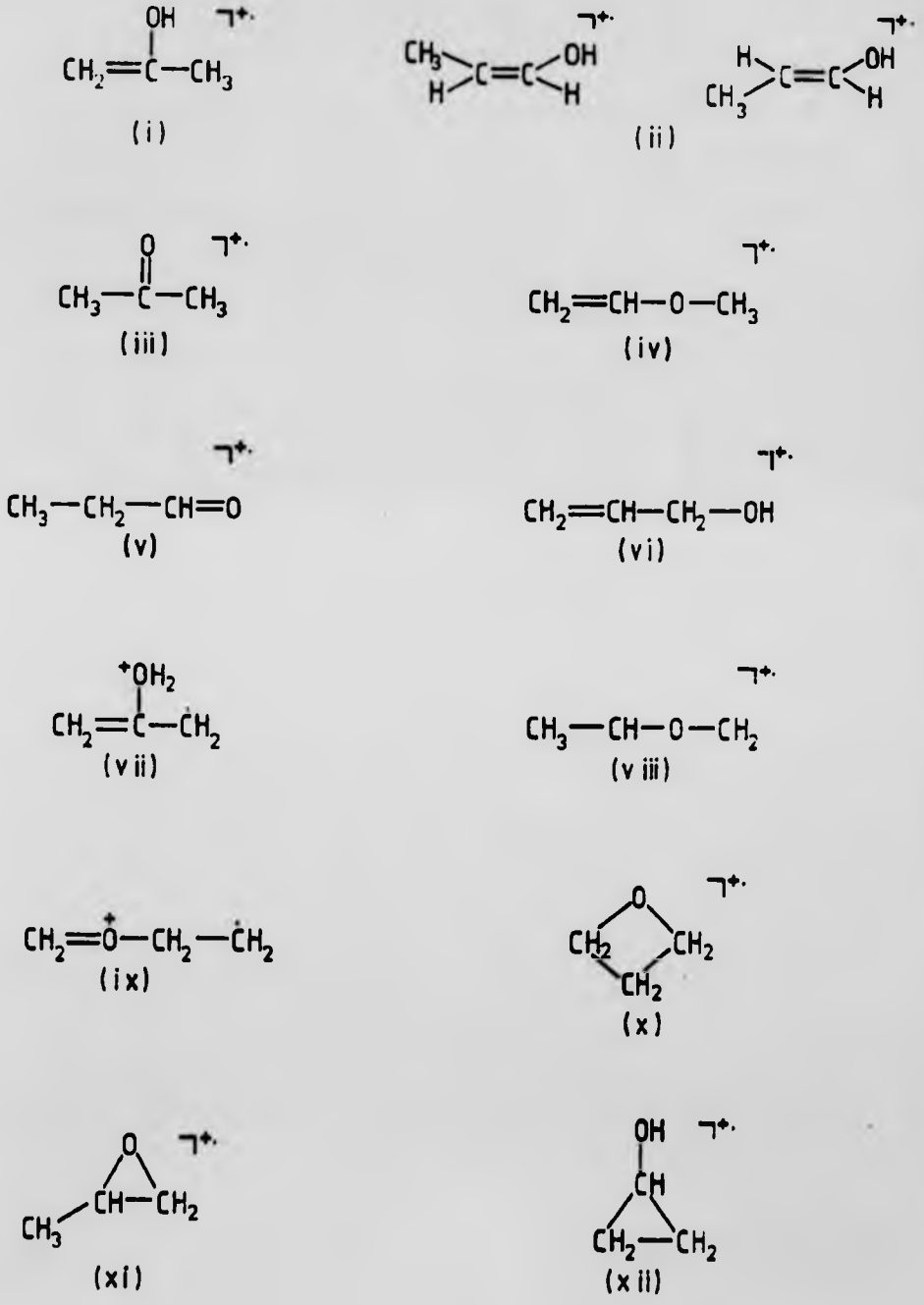
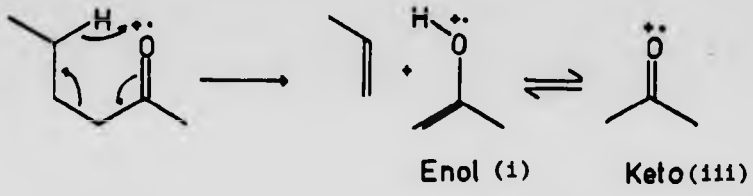


Figure 4.4. Structures of twelve isomeric $C_3H_6O^{+\cdot}$ ions.

4.3.2 *Historical Review of $C_3H_6O^{+\cdot}$ Data*

Initial interest in this field centred around the structure of the $C_3H_6O^{+\cdot}$ ion produced from various ketone molecular ions by either the single McLafferty rearrangement or the double McLafferty rearrangement, as shown in Figure 4.5(a) and (b) respectively.

(a) 2-Hexanone



(b) 5-Nonanone

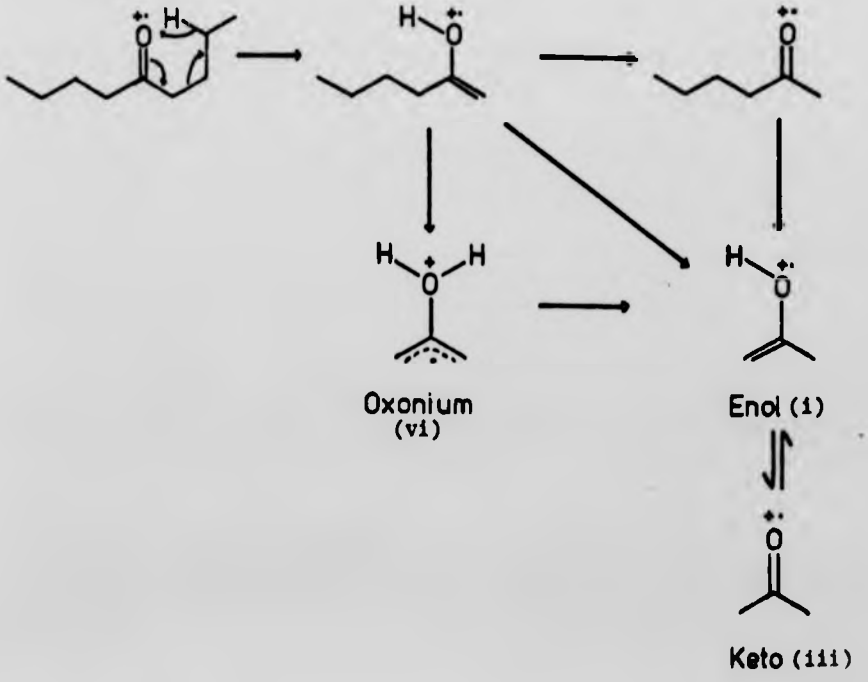


Figure 4.5. Single (a) and double (b) McLafferty rearrangements to give $C_3H_6O^{+\cdot}$ product ions.

Early experimental data[129,130] and calculations[129] led to the conclusion that the product of both the single and the double McLafferty rearrangements did not exist in the keto form (iii) and so probably had the enol structure (i). Metastable ion work[131] supported the conclusion that the keto and enolic isomeric forms could be distinguished. These results also suggested, however, that a substantial fraction of the $C_3H_6O^{+\cdot}$ ions formed by a double McLafferty rearrangement did not exist in the enolic form (i) and these ions probably existed with the oxonium ion structure (vii). In one of the first few studies of ionic structure elucidation by I.C.R.,[114,132] seven ion-molecule reactions were described which showed that the acetone radical cation (iii) and its enol isomer (i) were distinct, non-interconverting species. This, together with subsequent studies[133] involving deuterated ketones, showed that the products of the single and double McLafferty rearrangements existed in the enolic form with the product of the second rearrangement being formed directly and not by isomerisation from the oxonium ion or by ketonisation of the intermediate ion followed by hydrogen migration to the carbonyl oxygen. It has since been shown[128] that gas phase enolic ions are between 60 and 130 kJ $mole^{-1}$ more stable than their keto isomers with the acetone radical cation being 105 kJ $mole^{-1}$ less stable than its enolic form.

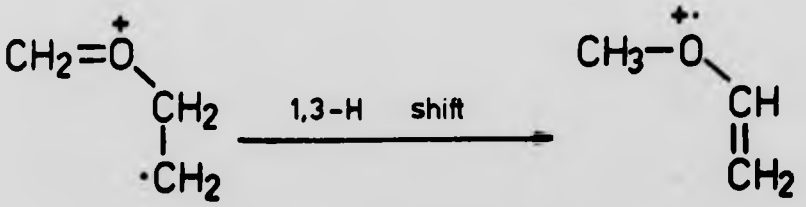
The discovery that metastable ion abundances were dependent upon internal energy,[78b] as well as structure, led to a re-evaluation of the oxonium ion data[134] followed by the subsequent withdrawal of this ion structure as a possible product ion of the double McLafferty rearrangement.[134] Furthermore and more importantly, this discovery led to the introduction of a technique with less dependence on internal energy, namely collisional activation.[123]

Thus the results of I.C.R. and C.A. techniques showed agreement in that the non-decomposing $C_3H_6O^{+\cdot}$ product ions of the McLafferty

rearrangement were formed with the enolic structure and did not subsequently isomerise. The hypothesis that decomposing $C_3H_6O^{+\cdot}$ ions with the enol structure do so via the keto structure has been the subject of several investigations.[123,134-136] No firm conclusions can be made about this point mainly because of the lack of control of the internal energy of the decomposing ions being sampled.

After the initial interest in the isomeric form of the $C_3H_6O^{+\cdot}$ ions produced by the McLafferty rearrangement attention was focussed on other $C_3H_6O^{+\cdot}$ isomeric forms. Analysis of the decomposition products of the metastable molecular ions of propylene oxide (xi), propanol (v) and trimethylene oxide (x) showed that they were distinct species.[137] In a C.A. study,[138] seven $C_3H_6O^{+\cdot}$ isomers were identified as distinct species, formed from a variety of precursors. They were the molecular ions of acetone (iii), propanol (v), vinyl methyl ether (iv), trimethylene oxide (x) and propylene oxide (xi), together with the enol isomers of acetone (i) and of propanol (ii). In the same investigation allyl alochol (vi) was reported to isomerise to the enol ion (ii), possibly by a 1,3 hydrogen shift, although such rearrangements usually have a high activation barrier.[139] One omission in this C.A. study was the cyclopropanol molecular ion (xii). Although the I.C.R. spectrum of cyclopropanol has been reported[140] the structure of the molecular ion has not been investigated. I.C.R.[99] and C.I.[103,107] studies of the vinyl methyl ether radical cation (iv) have identified ion-molecule reactions thought to be characteristic of this ion structure. A theoretical study[127] of seventeen $C_3H_6O^{+\cdot}$ isomers led to the suggestion that several isomers exist of lower energy than the characterised propylene oxide ion (xi) and also led to the investigation of the ring opened trimethylene acid ion (ix). A subsequent I.C.R. study[141] has identified a characteristic ion-molecule reaction for this structure ion formed from 1,4-dioxan.

In a metastable ion study, in which the metastable ion characteristics and the kinetic energy release of $C_3H_6O^{+\cdot}$ ions produced from 1,4-dioxan, trimethylene oxide, propylene oxide, propanol, vinyl methyl ether, allyl alcohol and acetone were reported, the results led to two conclusions. Firstly, that the dominant fraction of the decomposing $C_3H_6O^{+\cdot}$ ions from 1,4-dioxan had a structure similar to those of the molecular ion of trimethylene oxide. (This does not prove that the structures of these ions are cyclic as assumed in the C.A. study.[138] In the light of the I.C.R. study[141] the similarity could be accounted for in terms of ring-opened structures or a mixture of closed and open structures). Secondly, that there was little isomerisation of the $C_3H_6O^{+\cdot}$ ions produced from 1,4-dioxan and trimethylene oxide ions to other structures such as ionised acetone, allyl alcohol or vinyl methyl ether. The lack of tendency of the isomeric ring opened ion (ix) to isomerise to the vinyl methyl ether radical cation (iv) as shown in Figure 4.6, is not



(ix) (iv)

Figure 4.6. Isomerisation of isomer (ix) to isomer (iv) by a 1,3 H shift.

surprising considering the high energy of activation (~ 200 kJ mol^{-1}) needed for such reactions.[139] Finally, in two recent studies[136,142] it was shown that, in contrast to $C_4H_8O^{+\cdot}$ ions,[143] decomposing $C_3H_6O^{+\cdot}$ ions with the oxygen on the first carbon atom do not rearrange to their isomer with the oxygen on the second carbon atom and vice versa.

4.3.3 *Aims of this Study*

The aims of this I.C.R. study were as follows:-

1. To investigate the use of ion-molecule reactions for the characterisation of $C_3H_6O^{+\cdot}$ ions with the structure of the ionised vinyl methyl ether molecular ion (iv), $CH_3\text{-}O\text{-}CH{=}CH_2]^{+\cdot}$.

2. To investigate the use of ion-molecule reactions for the characterisation of $C_3H_6O^{+\cdot}$ ions with the structure of the ring opened trimethylene oxide molecular ion (ix), $CH_2{=}\overset{+}{O}\text{-}CH_2\text{-}\dot{C}H_2$.

3. To investigate the structure of the $C_3H_6O^{+\cdot}$ molecular ion generated from cyclopropanol.

4. To use the ion-molecule reactions from (1) and (2) to obtain information about the structure of $C_3H_6O^{+\cdot}$ fragment ions formed from a variety of precursors.

Where possible the results obtained have been compared with previously published work placing particular emphasis on a comparison with C.A. results.

4.4 $C_3H_6O^{+\cdot}$ ISOMERS - EXPERIMENTAL

All experiments were performed using a Varian V-5900 series I.C.R. mass spectrometer which has been described previously.[67,98,99] A four section "square" drift cell was used consisting of source, reaction, analyser and total ion current regions. Single resonance spectra were obtained using magnetic field modulation. The electron energy used was 13 eV except where stated. The trap current was in the range 50-100 nA, producing total ion currents of the order of 1-10 pA as measured by a Keithley 602 electrometer. The total sample pressures, as

indicated by a VIG10 ionisation gauge,(Vacuum Generators Limited) ranged from 2×10^{-6} to 6×10^{-4} Torr. Double resonance spectra were obtained using square wave modulation of the double resonance oscillator which was applied to the drift plates of the reaction region. The amplitude of the double resonance oscillator was kept as low as possible to try and avoid sweep out effects and spurious double resonance signals.[67]

With the exception of cyclopropanol, which was synthesised[144] by Mr B.W. Waters, all the chemicals used in the $C_3H_6O^{+\cdot}$ investigation were obtained from the following suppliers and were purified as necessary; Aldrich Chemical Company, Air Products, B.D.H. Chemicals Limited, Fisons, Fluka, Hopkin and Williams, Koch-Light Laboratories Limited, Mathesons, Merck Chemicals and Pfaltz and Baur Incorporated.

4.5 $C_3H_6O^{+\cdot}$ ISOMERS - RESULTS AND DISCUSSION

Due to space and time considerations, not all the ions observed in the large number of systems studied in this work will be discussed in detail. The I.C.R. spectra of many of the compounds studied have been reported previously. The relationship between the abundance of an ion in the I.C.R. spectrometer and the intensity of the peak representing that ion, recorded in the spectrum is not a simple one and hence relative intensities of the ions observed are not reported.

In general the fragment ions produced in the I.C.R. spectrometer correspond qualitatively to the major ions produced in an electron impact source. Most ion-molecule reactions in single component systems can be interpreted in terms of proton transfer to give an (MH^+) ion or cluster ion formation between a molecule and its molecular ion, protonated molecular ion (MH^+) or an abundant fragment ion. Such cluster or complex ions are frequently not observed in the spectrum

as they tend to lose appropriate neutral or radical species to form more stable ions. The tendency to lose stable, neutral molecular species such as CH_3-OH, H_2O, CH_2O from a complex often provides a driving force for an ion-molecule reaction to occur. As will be seen such behaviour is common in an I.C.R. spectrometer.

Ion-molecule reactions discussed will therefore be limited to those of relevance to the general theme of this work unless they are of particular interest for other reasons. All ion-molecule reactions discussed below have been substantiated by double resonance experiments unless stated otherwise.

4.5.1 *The Vinyl Methyl Ether Radical Cation, $CH_3-O-CH=CH_2]^{+\cdot}$*

In the following discussion the various resonance forms of the vinyl methyl ether radical cation, for example $CH_3-\overset{+\cdot}{O}-CH=CH_2$, $CH_3-\overset{+}{O}=CH-\dot{C}H_2$ and $CH_3-O-\overset{+}{C}H-\dot{C}H_2$, will be taken as being structurally equivalent and represented by the structure $CH_3-O-CH=CH_2]^{+\cdot}$ (iv).

By the use of mass spectrometry,[103] I.C.R.[99] and C.A.[107] experiments, facilitated by the use of deuterium (D) labelling, it has been shown that the V.M.E. molecular radical cation will:-

1. React with certain mono-olefinic compounds, as shown generally in Figure 3.1 and specifically in Figure 4.7 (a) and (b), to give products which are indicative of the position of the olefinic bond in the neutral compound.

2. React with conjugated dienes, as shown in Figure 4.7 (c), in a gas phase Diels-Alder reaction followed by the elimination of methanol.

Conversely, it should be possible to use olefins of known structure to test $C_3H_6O^{+\cdot}$ ions for the V.M.E. radical cation structure (iv).

In order to test this hypothesis the olefins 1-butene, 2-butene (*trans*) and 1,3-butadiene have been used.

Single Component Systems

The I.C.R. spectrum of V.M.E. has been reported previously.[99] The major ions observed in the single component systems ($P = 1 \times 10^{-5}$ Torr; 13 eV) are given below with the molecular ions of the various compounds being underlined.

V.M.E.	m/z	43, <u>58</u>, 59, 75, 84, 88, 101
1-Butene	m/z	41, 55, <u>56</u>, 57, 69, 70, 81, 82, 83, 84, 97
2-Butene	m/z	41, 55, <u>56</u>, 57, 69, 84
1,3-Butadiene	m/z	39, <u>54</u>, 55, 66, 67, 79, 80, 93

Two Component Systems

The major ion-molecule reactions observed in the systems (a) V.M.E./1-butene, (b) V.M.E./2-butene and (c) V.M.E./1,3-butadiene are given in Figure 4.7.

Product ions of m/z 86(58), 82(58), 67(41), 81(55), 45 and 72 (The figures in brackets represent the precursor ions as determined by double resonance.) are observed in the V.M.E./1-butene system in decreasing order of intensity. Ions of m/z 86 and 82 are probably formed *via* the reaction of 1-butene and the V.M.E. molecular cation (iv) followed by the loss of ethene and methanol respectively. Double resonance indicates that ions of m/z 67 and 81 are formed *via* the respective reaction of V.M.E. with m/z 41 (Butene - $CH_3\cdot$)$^+$ and m/z 55 (Butene - $H\cdot$)$^+$ followed by the loss of methanol in both cases. Product ions at m/z 72 and 45 were of too small an intensity to obtain double resonance signals although it is likely that 1-butene reacts with the V.M.E. radical cation to eliminate the ion $CH_3OCH_2^{+\cdot}$ (m/z 45), as this is a known reaction.[145]

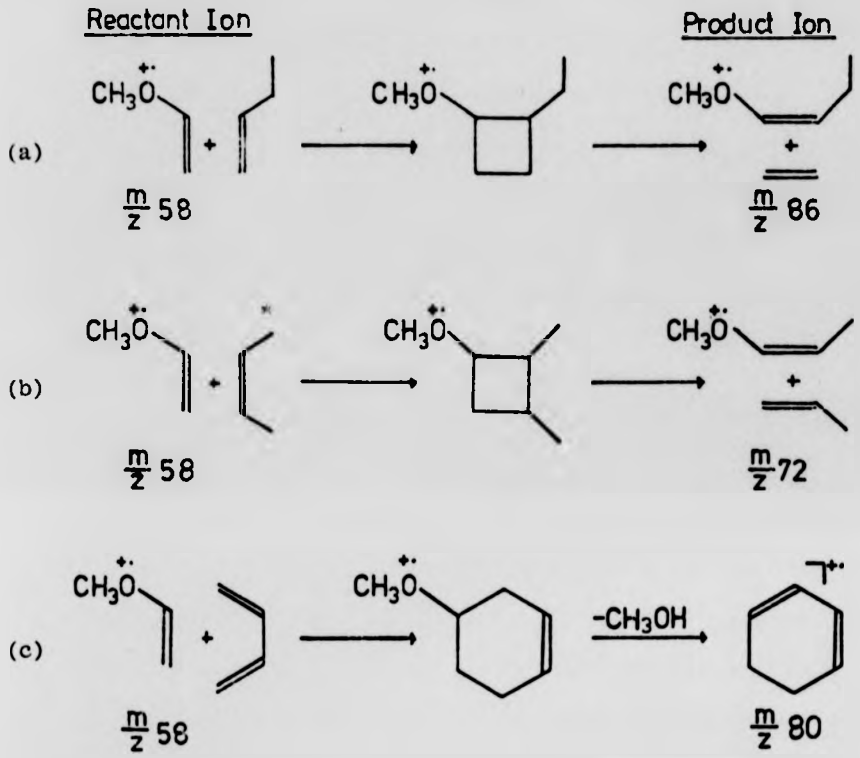


Figure 4.7. The major ion-molecule reactions observed in each of the systems (a) V.M.E. and 1-butene.
(b) V.M.E. and 2-butene.
(c) V.M.E. and 1,3-butadiene.
*cis form drawn here for convenience.

Product ions of m/z 72(58,56), 67(41), 81(55), and 45 are observed in the V.M.E./2-butene system. m/z 72 is most likely formed by the loss of propene from a complex formed between a 2-butene molecule and a V.M.E. radical cation. It is interesting to note that the double resonance spectrum of m/z 72 shows a small signal for m/z 56. A possible interpretation of this signal is that some of the m/z 72

ions are produced in the 2-butene/V.M.E. system in the same way as the m/z 72 ions are produced in the 1-butene/V.M.E. This reaction could therefore be viewed as a possible $CH_2^{+\cdot}$ transfer from the butene to the V.M.E. molecule. Product ions of m/z 67, 81 and 45 are most likely produced as explained above. A peak of very low intensity appears at m/z 82 indicating that the loss of methanol from the substituted cyclobutane complex is not as important in the case of 2-butene compared with 1-butene.

Figure 2.7 shows an I.C.R. spectrum of the V.M.E./1,3-butadiene system which has been investigated previously.[107] The double resonance spectrum of the product ion at m/z 80 is shown in Figure 2.9 indicating that both m/z 58 and m/z 54 are precursor ions. The single resonance spectrum of 1,3-butadiene shows a product ion at m/z 80(54) (see insert Figure 2.7) which is presumably formed owing to the loss of m/z 28 (C_2H_4) from a complex formed between a neutral and an ionised 1,3-butadiene molecule. It should be noted that in the single component system the product ion at m/z 80 is less intense than that at m/z 79. Hence the presence of an additional reaction to give m/z 80, as shown in Figure 4.7(c) in the case of V.M.E., can be detected by the enhancement of the m/z 80 peak intensity and by the presence of a signal in the double resonance spectrum other than for m/z 54. Ions at m/z 111(58), 97(58,54) and 81 are presumably given by the respective loss of $H^{\cdot}$, $CH_3^{\cdot}$ and $OCH_3^{\cdot}$ from the Diels Alder reaction complex.

During the study of these two component systems it was found that the optimum ratio of major product ion intensity to reactant ion intensity, $I_{86,72,80}:I_{58}$, occurred when the electron energy was in the range 11-13 eV, the total pressure was 2×10^{-5} Torr and the ratio of partial pressure $P_{olefin}:P_{V.M.E.}$ was between 1 and 2. The order of sensitivity (reactivity) of the test substrates was found to

be 1-butene > 1,3-butadiene > 2-butene. All three substrates give characteristic product ions of different mass in a convenient region of the spectrum. This is useful in case there is already an ion of mass 86, 72 or 80 in the spectrum of the compound under investigation.

Before using these test substrates it is necessary to investigate the possibility of any similar reaction occurring between the substrate and other $C_3H_6O^{+\cdot}$ isomers. For example isomer (vi) (CH_2=CH-CH_2-$\overset{+\cdot}{O}H$) might react with olefins in a manner similar to that of V.M.E. radical cations. At this stage, it is necessary to draw upon the knowledge of other studies in order to try and find neutral compounds that will give reference ions of known structures. The compounds used for this purpose have been listed in Table 4.3 and the reasons for their choice will now be given.

Isomer (i) has been shown to be formed by a single McLafferty rearrangement, as discussed previously, and therefore 2-hexanone has been chosen as a precursor for this ionic structure. Due to the lack of stability of vinyl alcohols, structure (ii) has not been investigated; a vinyl alcohol cannot therefore be used to produce a reference ion of structure (ii). 2-Methyl pentanal has been shown to give an intense $C_3H_6O^{+\cdot}$ ion, presumably *via* a McLafferty rearrangement and this is thought to have the structure of isomer (ii).[138] Acetone has been used to provide a reference ion of structure (iii).[114] The propanal molecular ion has been used as a reference ion for structure (v).[137] Although the allyl alcohol molecular ion has been shown in a C.A. study[138] to isomerise from structure (vi) to structure (ii) within 10^{-5} s, it may be that under the low energy conditions of I.C.R. the molecular ion will not isomerise. Therefore in the absence of any other precursor for ions of structure (vi) allyl alcohol has been used, although an open mind must be kept as to the structure of the molecular ion under I.C.R. conditions. No compounds giving ions of structure (vii)

or (viii) have been found yet, although there is slight evidence for some of the molecular ions of propylene oxide existing in the C-C ring-opened form, isomer (viii).[146] Isomer (ix) is the C-C ring-opened form of the trimethylene oxide molecular ion and has been shown to be produced from both trimethylene oxide and 1,4-dioxan.[127,141] The presence of ions of structure (x) formed from trimethylene oxide, cannot be proved or disproved because ions of this structure most likely react in the same way as those of the ring-opened structure (ix). Trimethylene oxide has therefore been used as a reference compound for isomer (ix)/(x) and 1,4-dioxan has been used as a reference compound for isomer (ix) only. Propylene oxide has been used as a precursor for isomer (xi)[138] and in the absence of any evidence to the contrary (see Section 4.5.3) cyclopropanol has been used as a precursor for isomer (xii).

Table 4.4 lists the results of the investigation of the two component systems involving the V.M.E. test substrates and the reference compounds.

From Table 4.4 it can be seen that ion-molecule reaction products of m/z 86, 72 and 80 are produced only in the systems involving V.M.E. Reaction products are observed in several of the systems listed in Table (4.4). Except for the systems involving propylene oxide, double resonance signals could not be obtained for these product ions, due to their low abundance. The most likely explanation of the ions of m/z 81 and 82 formed in the propylene oxide systems is that they are produced *via* the reaction of a neutral propylene oxide molecule with the olefin molecular ions followed by the loss of H_2O and $CH_3^{\cdot}$ in the case of the butenes or a formaldehyde molecule in the case of the diene; as shown in equations 4.3, 4.4 and 4.5.

Table 4.4. Reaction Products (precursor ions in brackets) observed in the two component systems involving 1-butene, 2-butene, 1,3-butadiene and the reference compounds.

Reference Compound	Ion Structure	1-butene	2-butene	1,3-butadiene
2-Hexanone	i	-	-	-
3-Methyl Pentanal	ii	-	-	99
Acetone	iii	-	-	-
V.M.E.	iv	86(58)	72(58)	80(58,54)
Propanol	v	-	66, 68	-
Allyl Alcohol	vi/ii	-	-	-
1,4-Dioxan	ix	-	-	-
Trimethylene Oxide	ix(x)	-	-	-
Propylene Oxide	xi	81(56)	81(56)	82(54)
Cyclopropanol	xii	69	69	68

- indicates no reaction product(s) detected in these systems.

$$\text{Olefin}^{+\cdot} + \text{Propylene Oxide} \rightarrow \text{Complex} \rightarrow \text{Observed Ion} \qquad 4.3$$

$$56^{+\cdot} + 58 \longrightarrow (\underline{114}) \xrightarrow{-33(H_2O,CH_3)} 81^{+} \qquad 4.4$$

$$54^{+\cdot} + 58 \longrightarrow (\underline{112}) \xrightarrow{-30(CH_2O)} 82^{+\cdot} \qquad 4.5$$

(–) indicates that this ion is not observed in the spectrum.

The mechanism of these reactions has not been investigated.

4.5.2 *The C-C Ring-Opened Trimethylene Oxide Cation, $CH_2=\overset{+}{O}-CH_2-\dot{C}H_2$*

By the use of I.C.R.[141] and D-labelling it has been shown that the $C_3H_6O^{+\cdot}$ ions formed from 1,4-dioxan by the elimination of formaldehyde from the molecular ions, will transfer a $C_2H_4^{+\cdot}$ species to acetonitrile and do not undergo ring closure prior to this transfer.[141] See Figure 4.8..

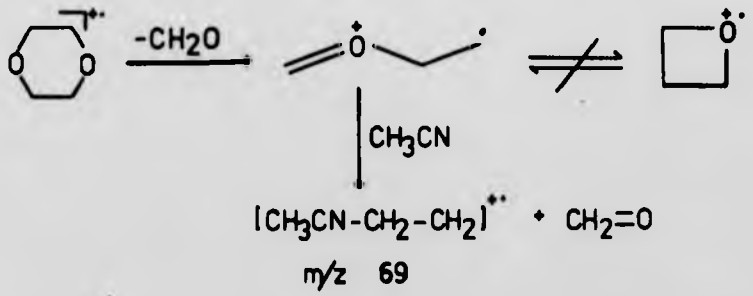


Figure 4.8. Schematic of the mechanism of production of the ion m/z 69 in the 1,4-dixoan/acetonitrile system.

A similar $C_2H_4^{+\cdot}$ transfer has been observed in the case of trimethylene oxide and acetonitrile, indicating the presence of ions of structure (ix), although the product peak is weaker compared with 1,4-dioxan. However, the fact that ions of structure (x) may react similarly or that ions of structure (x) are present in ionised trimethylene oxide cannot be ruled out.

In order to make use of this $C_2H_4^{+\cdot}$ transfer reaction, acetonitrile and acrylonitrile have been used as test substrates.

Single Component Systems

The major ions observed in the single component systems [P = 1 x 10^{-5} Torr; 13 eV] are given below.

Acetonitrile	m/z	40, **41**, 42, 54, 68, 83
Acrylonitrile	m/z	**53**, 54, 79
Trimethylene Oxide (T.M.O.)	m/z	28, 29, 30, 31, 41, 57, **58**, 59, 71, 73, 87, 89, 99, 115, 117
1,4-Dioxan	m/z	44, 45, 57, 58, 87, **88**, 89.

Two Component Systems

As expected the only product ions observed in the systems (A) Acetonitrile/(1) T.M.O. (2) 1,4-dioxan and (B) Acrylonitrile/(1) T.M.O. (2) 1,4-dioxan were at m/z 69 for systems (A)(1) and (2) and at m/z 81 for systems (B)(1) and (2). In every case the double resonance spectra of the ions of m/z 69 and 81 gave signals for m/z 58 only, indicating the occurrence of the reactions shown in equations 4.6 and 4.7.

$$\underset{\text{m/z 58}}{CH_2=\overset{+}{O}-CH_2-\dot{C}H_2} + CH_3CN \longrightarrow CH_2O + \underset{\text{m/z 69}}{(C_2H_4\cdot CH_3CN)^{+\cdot}} \qquad 4.6$$

$$\underset{\text{m/z 58}}{CH_2=\overset{+}{O}-CH_2-\dot{C}H_2} + CH_2=CHCN \longrightarrow CH_2O + \underset{\text{m/z 81}}{(C_2H_4\cdot CH_2=CHCN)^{+\cdot}} \qquad 4.7$$

As with the olefin substrates the possibility of a similar ($C_2H_4^{+\cdot}$) transfer to the nitrile substrates from isomers of structure other than (ix/x) was checked using the same reference compounds. The results of this investigation are listed in Table 4.5.

Table 4.5. Reaction Products (precursor ions in brackets) observed in the two component systems involving acetonitrile or acrylonitrile and the reference compounds.

Reference Compound	Ion Structure	CH_3CN	C_2H_3CN
2-Hexanone	i	83, 99	91, 112
3-Methyl Pentanal	ii	-	-
Acetone	iii	84(58)	96(58)
V.M.E.	iv	-	-
Propanol	v	-	-
Allyl Alcohol	vi/ii	82(42)	94(54)
1,4-Dioxan	ix	69(58)	81(58)
Trimethylene Oxide	ix/x	69(58) 82, 100	81(58), 94, 112(59,54)
Propylene Oxide	xi	82(59)	94(54)
Cyclopropanol	xii	-	-

- indicates no reaction product(s) detected in these systems

From Table 4.5 it can be seen that ion-molecule reaction products of m/z 69. in the case of acetonitrile, and m/z 81 in the case of acrylonitrile, are formed only in the systems involving 1,4-dioxan or trimethylene oxide. Reaction products are observed in several of the systems listed in the table. Some of these product ions are either of too low an intensity to give double resonance signals or else the double resonance spectra contain several peaks indicating a variety of precursor ions. The results obtained for propylene oxide indicate the occurrence of the reaction sequences shown in equations 4.8 and 4.9.

$$59^+ + \underset{(CH_3CN)}{41} \longrightarrow (100) \xrightarrow{-18(H_2O)} 82 \qquad 4.8$$

$$58 + \underset{(C_2H_3CN)H^+}{54^+} \longrightarrow (112) \xrightarrow{-18(H_2O)} 94 \qquad 4.9$$

It is interesting that although a similar reaction occurs in both systems the protonated propylene oxide ion is involved in the case of acetonitrile and the protonated acrylonitrile ion is involved in the latter case. These reactions were not investigated further.

The product ions m/z 82 and 94 observed in the allyl alcohol systems gave double resonance signals for m/z 42 and 53 respectively indicating the occurrence of similar reaction sequences as shown below.

$$\underset{\text{Allyl Alcohol}}{58} + \underset{(CH_3CN)H^+}{42^+} \longrightarrow (\underline{100})^+ \xrightarrow{-18(H_2O)} 82^+ \qquad 4.10$$

$$58 + \underset{(C_2H_3CN)H^+}{54^+} \longrightarrow (\underline{112})^+ \xrightarrow{-18(H_2O)} 94^+ \qquad 4.11$$

It was not possible to obtain useful double resonance spectra for the product ions formed in the 2-hexanone systems or for the product ions formed in the trimethylene oxide systems.

Several ion-molecule reactions have already been shown to be characteristic of the acetone molecular cation structure (iii) and the results given in Table 4.6 indicate the presence of another such reaction. The product ions m/z 84 and 96 produced in the acetone/acetonitrile and acetone/acrylonitrile systems respectively both gave double resonance signals for m/z 58 only, indicating the occurrence of the following reactions.

$$58^{+\cdot} + 41 \longrightarrow (\underline{99}) \xrightarrow{-15(CH_3^{\cdot})} 84^{+} \qquad 4.12$$

$$58^{+\cdot} + 53 \longrightarrow (\underline{111}) \xrightarrow{-15(CH_3^{\cdot})} 96^{+} \qquad 4.13$$

This reaction was investigated more fully. In order to find out from where the methyl radical group was lost and how general the reaction was, a variety of ketones and nitriles (cyanides) were studied as shown in Table 4.6. The results are summarised in Figure 4.9 and show that this reaction occurs for small ketones with acetonitrile and a variety of nitriles with acetone. Acetaldehyde and the ketones 2-pentanone, 2-hexanone and 2-heptanone do not produce an analogous reaction.

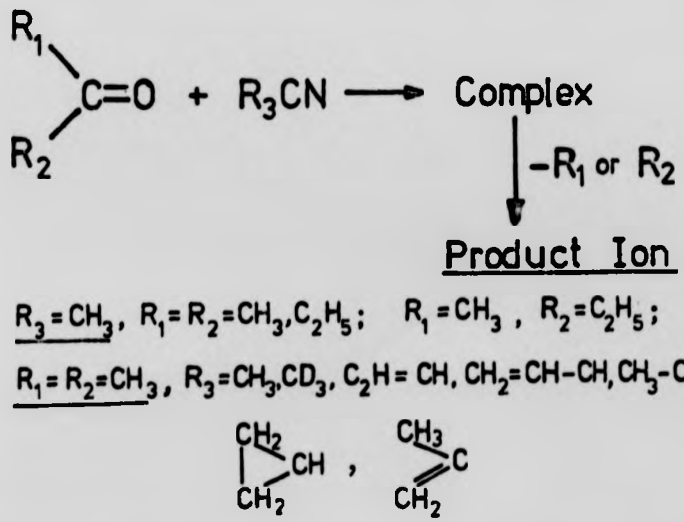


Figure 4.9. Summary of the compounds that undergo the general ion-molecule reaction shown above.

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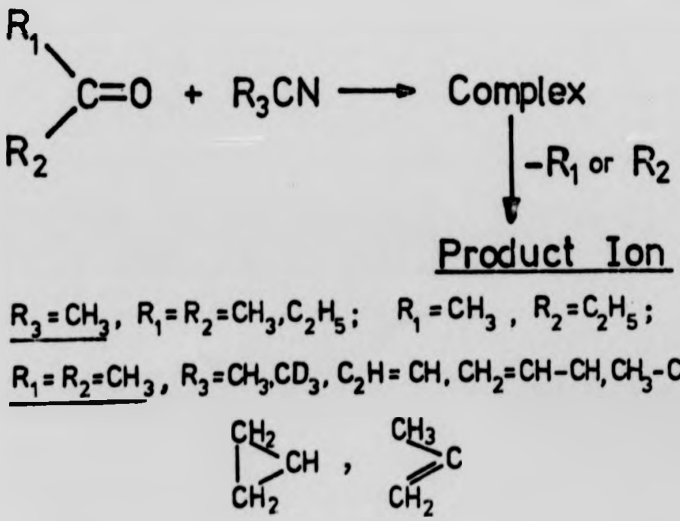


Figure 4.9. Summary of the compounds that undergo the general ion-molecule reaction shown above.

Table 4.6. Summary of the ketone / nitrile systems studied in the I.C.R. spectrometer.

	Ketones	Cyanides	Product Ions
	Acetaldehyde (44)	CH_3CN	86(45,42), 84
	Acetone (58)	CH_3CN	84(58)
	Acetone (58)	CD_3CN	87(58)
	Acetone (58)	C_2H_3CN	97(58)
	Acetone (58)	C_4H_5CN	110(58), 126(59)
	Acetone (58)	C_4H_5CN	110(58), 126(59)
	Acetone (58)	C_4H_5CN	110(58), 126(59)
	2-Butanone (72)	CH_3CN	84(72), 98(72)
	3-Pentanone (86)	CH_3CN	98(86)
	2-Pentanone (86)	CH_3CN	99(86), 128(87,86)
	2-Hexanone (100)	CH_3CN	83, 99
		C_2H_3CN	91, 112
	2-Heptanone (114)	CH_3CN	97
		C_2H_3CN	—, —

4.5.3 *The Cyclopropanol Molecular Ion*

The structure of the cyclopropanol molecular ion has not been studied extensively although the I.C.R. spectrum of cyclopropanol has been reported.[140] If the cyclopropanol molecular ion ring opens after ionisation by electron impact then it may exist in one of the forms shown in Figure 4.10.

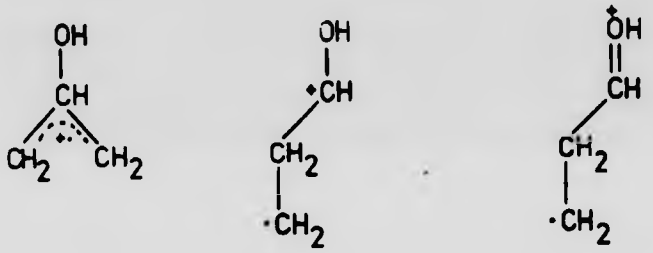


Figure 4.10. Possible structural forms of the ring opened cyclopropanol molecular ion.

If this is the case, then the ring opened ion may transfer a $CH_2^{+\cdot}$ group to a suitable neutral acceptor leaving the neutral alcohol CH_2=CH-OH. Nitrile compounds have been shown to be good acceptors of $CH_2^{+\cdot}$[147] and $C_2H_4^{+\cdot}$[148] groups. In the I.C.R. spectrum of cyclopropanol and acrylonitrile there was no sign of such a transfer occurring. Therefore due to lack of evidence to the contrary it must be assumed that the cyclopropanol molecular ion does not ring open after formation by low energy electron impact.

4.5.4 *The Structure of Various $C_3H_6O^{+\cdot}$ Fragment Ions*

The substrates 1-butene, 2-butene, 1,3-butadiene, acetonitrile and acrylonitrile, examined in Sections 4.5.1 and 4.5.2 were used to test $C_3H_6O^{+\cdot}$ fragment ions formed from a variety of precursors for the structures CH_3-O-CH_2=$CH_2]^{+\cdot}$ (iv) and CH_2=$\overset{+}{O}$-CH_2-$\dot{C}H_2$ (ix). The results of these studies are summarised in Table 4.7.

Table 4.7. Summary of the product ions observed when the compounds (1-7) were examined together with the olefin and nitrile substrates.

COMPOUND	PRODUCT IONS OBSERVED				
	1-Butene 86(58)	1,3-Butadiene 80(58,54)	2-Butene 72(58)	Acetonitrile 69(58)	Acrylonitrile 81(58)
1. 2-Methoxyethyl-acetate	YES	YES	YES	NO	NO
2. 2-Methoxyethyl-acrylate	YES	YES	YES	NO	NO
3. 1,2-Dimethoxyethane	YES	YES	-	69(60)	81(60)
4. 1-Chloro-3-methoxy-ethane	YES	YES	NO	NO	NO
5. 2-Methoxyethanol	NO	NO	NO	YES	YES
6. 1,2-Epoxyoctane	NO	NO	NO	NO	NO
7. 3-Chloropropanol	NO	NO	NO	YES	YES

If a compound of the form $CH_3O-CH_2-CH_2-X$ loses HX after ionisation then it may do so *via* a 1,2 or 1,4 elimination, forming $C_3H_6O^{+\cdot}$ ions of isomeric structure (iv) and (ix) respectively, as shown in Figure 4.11.

Isomer (iv) may be formed *via* a 4-centre transition state with the elimination of HX involving a methylene hydrogen.

Isomer (ix) may be formed *via* a 6-centre transition state with the elimination of HX involving a methyl hydrogen.

Isomer (ix) may be formed directly or via the formation of a ring, isomer (x) which may subsequently ring open. Whilst a 6 membered transition state is more energetically favourable than a 4 membered transition state, it is also more favourable to transfer a methylene

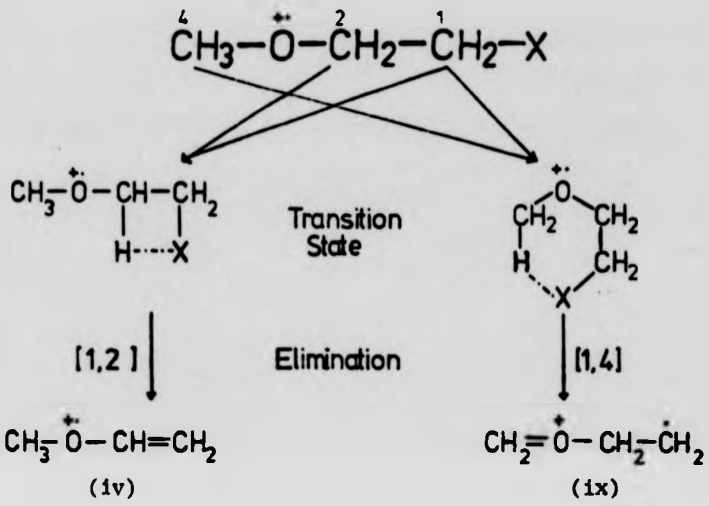


Figure 4.11. [1,2] and [1,4] elimination of HX from substituted methoxyethane molecular ions.

hydrogen than a methyl hydrogen. Hence the choice between a [1,2] and a [1,4] elimination of HX is finely balanced and the predominant pathway will be determined by the nature of the substituent X. The olefin and nitrile substrates were used to test for the occurrence of [1,2], [1,4] or both types of elimination of HX for the cases where

$$X = O\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_3,\ OCH{=}CH_2,\ OCH_3,\ Cl \text{ and } OH.$$

The results, briefly summarised in Table 4.7, 1-5, will now be discussed in more detail.

<u>2-methoxy acetate and 2-methoxy acrylate</u> were chosen to be studied first because they have been shown to give rise to $C_3H_6O^{+\cdot}$ isomers of the V.M.E. radical cation structure (iv).[138] The I.C.R. results confirm this, but it should not be assumed that this implies a [1,2] hydrogen transfer involving a 4 membered transition state. It is more likely

that a [1,4] hydrogen transfer of the methylene hydrogen to the carbonyl oxygen in the case of the acetate or the terminal methylene group in the case of the acrylate occurs. In this case the [1,2] elimination proceeds through a 6 membered transition state as shown in Figure 4.12.

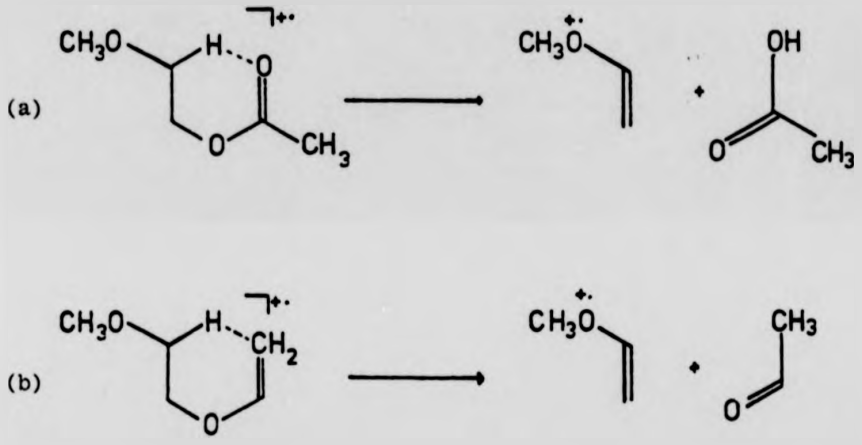


Figure 4.12. [1,2] elimination of (a) CH_3COOH from 2-methoxy acetate and (b) CH_3CHO from 2-methoxy acrylate *via* a [1,4] H shift.

1,2-Dimethoxyethane shows evidence of a predominant [1,2] elimination of methanol, Figure 4.13(a). This agrees with a previous I.C.R. study[149] which reported 94% loss of CH_3OD from CH_3-O-CD_2-CD_2-O-CH_3 (2-Butene could not be used as a test substrate due to the presence of an ion-molecule reaction product at m/z 72 in the 1,2-dimethoxyethane single compound system. This product ion gave a signal for m/z 58 both with and without 2-butene present.). In the case of 1,2-dimethoxyethane and acetonitrile/acrylonitrile, product ions were observed at m/z 69 and 81 respectively and double resonance experiments indicated that ions of m/z 60 were precursors of these product ions. It has been shown previously[149] that the ion of m/z 60 in the 1,2-dimethoxyethane spectrum has the oxonium ion structure, as shown in Figure 4.13(d)

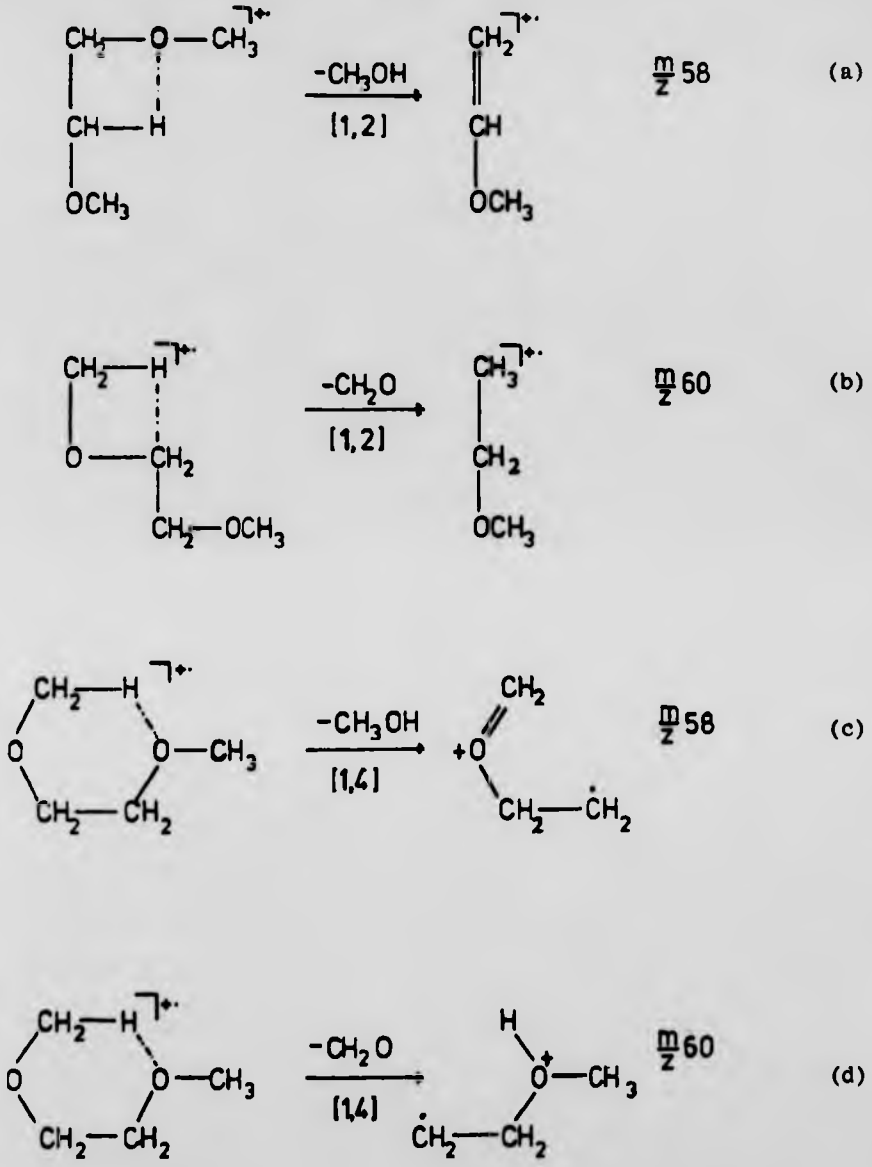


Figure 4.13. Possible routes to the formation of ions of m/z 58 and 60 in the I.C.R. spectrum of 1,2-dimethoxyethane.

and that it will transfer $C_2H_4^{+\cdot}$ to nitrile compounds.[148] Thus it appears as if methanol is lost from the molecular ion of 1,2-dimethoxyethane in a [1,2] elimination as shown in Figure 4.13(a) in preference to a [1,4] elimination as shown in Figure 4.13(c). Furthermore, formaldehyde appears to be lost in a [1,4] elimination, Figure 4.13(b). Thus reaction (a) (Figure 4.13) occurs in preference to reaction (b) and reaction (d) occurs in preference to (c). The preferential loss of methanol or formaldehyde from the 4 or 6 membered transition states is most likely to be determined by the relative heats of formation of the product ions.

The I.C.R. spectrum of 1-chloro-2-methoxyethane shows very weak peaks due to product ions at m/z 86 and 80 in the presence of 1-butene and 1,3-butadiene respectively. Double resonance confirms m/z 58 as being a precursor ion in both cases. No product ion of m/z 72 was observed in the 1-chloro-2-methoxyethane/2-butene system. 1-chloro-2-methyoxyethane showed no sign of eliminating HCl in a [1,4] manner to give $C_3H_6O^{+\cdot}$ ions of structure (ix). HCl is usually lost in a [1,3] elimination[150] and the results of a C.A. study[138] of 3-chloro-1-propanol indicated the loss of HCl in both [1,3] and [1,4] eliminations in the ratio of 2:3 at 70 eV and 20 eV. The [1,4] elimination to give $C_3H_6O^{+\cdot}$ ions of structure (ix) (or structure (x)) and the lack of [1,2] elimination to give ions of structure (iv) was confirmed by I.C.R., see Table 4.3 no. 7. The [1,3] elimination could not be confirmed by I.C.R. In the light of these results it seems surprising that 1-chloro-2-methoxyethane should not appear to lose HCl in a [1,4] elimination, especially when the loss of HCl is considered in terms of bond energy. 1-Chloro-2-methoxyethane would be expected to lose HCl in a [1,4] elimination more readily than 3-chloropropanol because the bond energy of -O-H is slightly greater than that of $-H_2C-H$. If the carbon-oxygen chain of the two molecular ions is examined then there should be no

real differences between the flexibility of the molecular ions of 1-chloro-2-methoxyethane and 3-chloropropan-1-ol because they both contain three carbon and one oxygen atoms. Thus further experiments are needed to clarify this result. It should be noted that the reaction between $C_3H_6O^{+\cdot}$ ions formed from 1-chloro-2-methoxyethane and 1-butene or 1,3-butadiene is weak and is non existent in the case of 2-butene. It may be that the ions of structure (iv) produced by a [1,2] elimination from 1-chloro-2-methoxyethane are formed in a high internal energy state.

2-methoxyethanol was the only compound examined in this series in which the molecular ion showed evidence of a [1,4] elimination of HX. It is well known[151] that long carbon chain alcohols prefer to lose water predominantly by a [1,4] elimination with [1,3] and [1,5] eliminations being the next most favoured routes. The results of a C.A. study[138] concluded that the $C_3H_6O^{+\cdot}$ ions formed from 2-methoxyethanol had the structures (iv) and (ix) in the ratios 1:5 at 70 eV and 3:7 at 20 eV. From I.C.R. experiments, no evidence could be found for the presence of ions of structure (iv). This discrepancy could be accounted for in several ways. The most likely explanation is that any $C_3H_6O^{+\cdot}$ ions formed by [1,2] elimination, which is not a common route of H_2O loss in alcohols, may be formed with too high an internal energy to react with the olefin substrates.

1,2-Epoxyoctane was examined in the I.C.R. instrument because a C.A. study[138] had shown the presence of $C_3H_6O^{+\cdot}$ ions of isomeric structure (iv) as well as those of the ionised propylene oxide structure (xi). The C.A. results indicated that at an electron energy of 70 eV the structures (iv) and (xi) were formed in the ratio 4:1 but at 20 eV $C_3H_6O^{+\cdot}$ ions of structure (iv) only were produced. The I.C.R. results showed that ions of structure (iv) could not be detected.

A mass spectrometric study of terminal epoxides[152] suggests that

the $C_3H_6O^{+\cdot}$ ions are formed with structure (vi), although an alternative pathway forming ions of structure (xi) has also been suggested.[153] These two routes are shown in Figure 4.14.

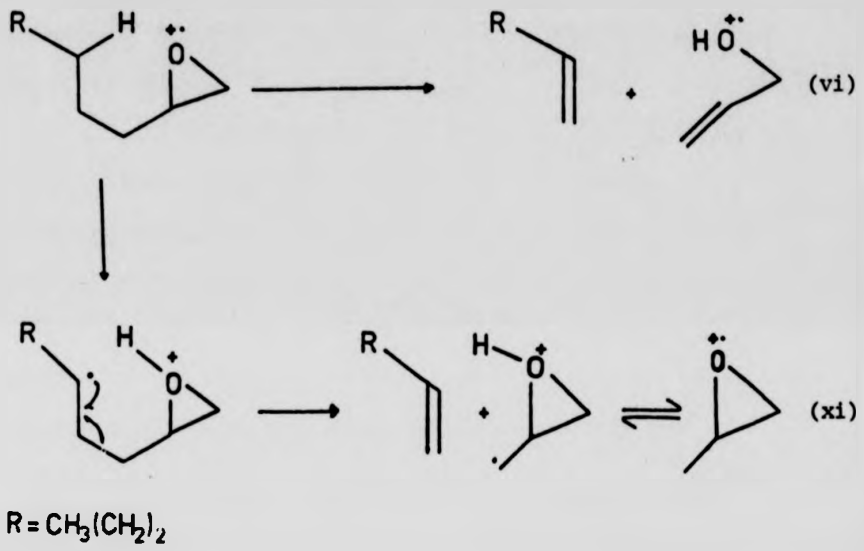


Figure 4.14. Possible pathways to the formation of $C_3H_6O^{+\cdot}$ ions from terminal epoxide molecular ions.

In view of these results and the fact that in the C.A. study[138] no reference ion for structure (vi) was found it must be concluded that the most likely structures for the $C_3H_6O^{+\cdot}$ ions formed from 1,2-epoxyoctane in the I.C.R. spectrometer are structure (vi) and (xi).

4.6 $C_3H_6O^{+\cdot}$ ISOMERS - CONCLUSIONS AND FURTHER WORK

The results presented in this chapter illustrate how the study of ion-molecule reactions using I.C.R. can be applied to determine ion structure. Three ion-molecule reactions have been used to ascertain the structure of various $C_3H_6O^{+\cdot}$ ions formed in gas phase elimination reactions. The results obtained demonstrate that this technique provides alternative and complementary information to that of

collisional activation. This technique can also be used in place of D-labelling experiments to ascertain the position of preferential hydrogen loss in an ion although the data obtained is only qualitative. The discrepancies in the results of this work and C.A. work illustrate the care that is needed in interpreting the results of either experiments in terms of internal energy effects, isomerisation in the case of C.A. and extent of reaction in the case of I.C.R., and the need for reference ions of known structure. Although C.A. can be used to sort out mixtures of structures on a semi-quantitative basis, this can be misleading if two structurally different ions give rise to similar C.A. spectra. The I.C.R. method gives a firm yes/no answer to the presence of a particular ion structure but does not allow an estimate of the quantity of that particular isomer to be ascertained.

Finally, several future experiments might prove worthwhile. It would be interesting to look at 2-methoxyethanol at higher electron energies to see if $C_3H_6O^{+\cdot}$ ions of structure (iv) were formed. (The elimination of HCl needs to be examined using a wider range of compounds.) If the deuterium labelled compound $CD_3-O-CH_2-CH_2-OD$ could be synthesised and examined in the I.C.R. instrument in the presence of acetonitrile it would be possible to see if the ring opened trimethylene oxide ion, structure (x) was formed after the loss of H_2O. This would allow the two reaction paths shown in Figure 4.15 to be distinguished.

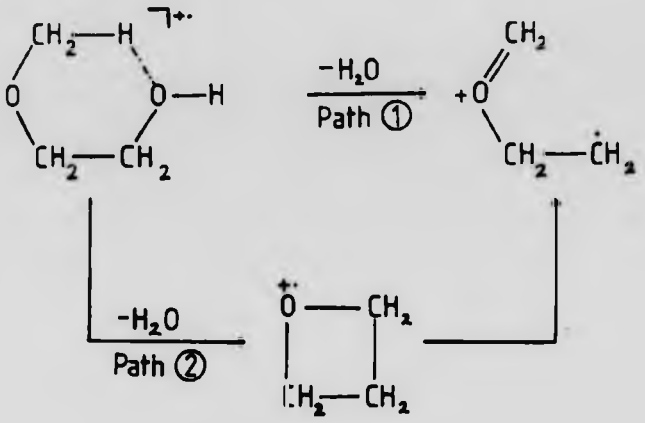


Figure 4.15. Two possible routes to the formation of ion (ix) from the 2-methoxyethanol molecular ion.

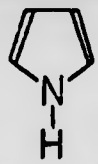
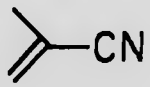
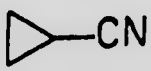
CHAPTER 5

ION-MOLECULE REACTIONS OF SOME $C_4H_5N^{+\cdot}$ ISOMERS

5.1 INTRODUCTION

An I.C.R. study has been made of the isomeric $C_4H_5N^{+\cdot}$ ions derived from five different neutral compounds of molecular formula C_4H_5N. These compounds are listed in Table 5.1 together with their ionisation energies,[154] where these are known.

Table 5.1. The five compounds used in this study to generate $C_4H_5N^{+\cdot}$ ions.

Neutral	Structure	I.E.(eV)[154]
1. Pyrrole		8.21
2. Allyl Cyanide		10.22
3. Crotyl Cyanide		-
4. Methacrylonitrile		10.34
5. Cyclopropyl Cyanide		10.20

The ion-molecule reactions of the smaller alkyl cyanides have long been of interest owing to the possibility of their involvement in the synthesis of organic systems in primitive atmospheres.[155] However, the present work was undertaken for more fundamental reasons. $C_4H_5N^{+\cdot}$ ions are isoelectronic with $C_4H_4O^{+\cdot}$ ions. It was the uncertainty over the structure of $C_4H_4O^{+\cdot}$ ions,[156] formed from various precursors, which led Sakurai and Jennings to study the metastable transitions and collision

induced decompositions of $C_4H_5N^{+\cdot}$ ions formed from eleven different neutral compounds. The work reported in this chapter constitutes an attempt to confirm some of the results of Sakurai and Jennings.[157]

Concentrating upon the data measured using the five compounds of interest in this study (Figure 5.1), it was found[157] that the only peak of any significance in the (B/E) metastable ion spectra of the molecular ions $C_4H_5N^{+\cdot}$ (m/z 67) was at m/z 41. High resolution mass spectrometric studies involving pyrrole,[159] together with thermochemical data[154] and PIPECO studies[154] involving pyrrole, methacrylonitrile, allyl cyanide and cyclopropyl cyanide indicate that the fragment ion of m/z 41 is the $C_2H_3N^{+\cdot}$ ion rather than the $C_3H_5^{+}$ ion. The amount of translational energy released in this decomposition, $[C_4H_5N^{+\cdot}] \longrightarrow C_2H_3N^{+\cdot}$ and C_2H_2, see Table 5.2, was found to lie in

Table 5.2. Kinetic energy release data and appearance energy data for the transition m/z 67 → 41.

Compound	m/z 67 ($C_2H_5N^{+\cdot}$) ⟶ m/z 41 ($C_2H_3N^{+\cdot}$)	
	Kinetic Energy[157] Released (meV)	Appearance Energy[154] (m/z 41) (eV)
1. Pyrrole	15.2	11.75
2. Allyl Cyanide	15.6	11.10
3. Crotyl Cyanide	19.1	-
4. Methacrylonitrile	22.1	11.65
5. Cyclopropyl Cyanide	15.1	11.00

the range 19.5 ± 4.5 meV.[157] This variation in kinetic energy was thought not to be structurally significant but due to internal energy effects and hence the conclusion was made that the decomposing $C_4H_5N^{+\cdot}$ ions produced from pyrrole, allyl cyanide, crotyl cyanide, methacrylonitrile and cyclopropyl cyanide isomerise to a common structure or

mixture of structures prior to decomposition. Supporting this conclusion is the fact that the appearance energy for the formation of $C_2H_3N^{+\cdot}$ ions from $C_4H_5N^{+\cdot}$ ions, for the compounds pyrrole, allyl cyanide, methacrylonitrile and cyclopropyl cyanide, is similar and falls in the range 11.40 ± 0.35 eV. Also, in a collisional activation and metastable ion study of the structure of $C_2H_3N^{+\cdot}$ ions[158] it was concluded that such ions had the same structure when they were formed from the five precursors of interest in this study. One possible explanation for this could be that the $C_2H_3N^{+\cdot}$ ions were formed from precursor ions of a common structure or common mixture of structures.

The results of Sakurai and Jennings[157] are in agreement with a photoion-photoelectron coincidence (P.I.P.E.C.O.) study of allyl cyanide, methacrylonitrile, cyclopropyl cyanide and pyrrole. In this study[154] it was found that the molecular ions of these four compounds (crotyl cyanide was not studied) rearranged to a common structure prior to decomposition. The common structure was thought to be like the "pyrrole molecular ion" as this was the lowest energy isomer. This conclusion is only valid in the metastable energy range because at higher energies the fragmentation rates may become competitive with those of isomerisation.[160]

Indeed, examination of the collisional activation (C.A.) spectra of $C_4H_5N^{+\cdot}$ ions implies that the non-decomposing $C_4H_5N^{+\cdot}$ ions, formed from the five neutral precursors of interest in this work, fall into three structurally distinct groups. These three groups are shown in Figure 5.1 according to the structure of the precursor compounds.

Ion cyclotron resonance (I.C.R.), like C.A., samples non-decomposing ions and so provides a complementary technique for ion structure determination. Thus the aim of this study was to find suitable ion-molecule reactions that would confirm or contradict the

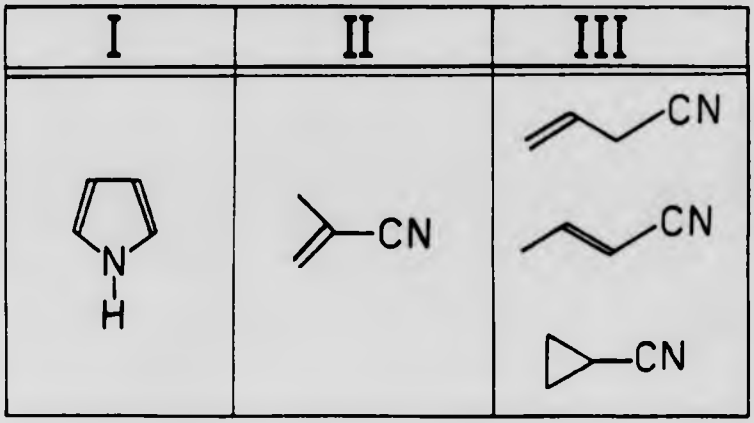


Figure 5.1. Structural categorisation of the C.A. spectra of $C_4H_5N^{+\cdot}$ ions according to the structure of the neutral precursors.

C.A. results. Hopefully this would then not only allow the categorisation shown in Figure 5.1 to be substantiated, but also allow the actual ionic structures of the various $C_4H_5N^{+\cdot}$ ions to be identified more clearly.

5.2 EXPERIMENTAL

The experimental conditions were the same as those used in the $C_3H_6O^{+\cdot}$ study (see Section 4.4). With the exception of ethyne, all the compounds were obtained from commercial sources (see Section 4.4) and were used without further purification. Ethyne was prepared and collected in a simple vacuum line using the reaction of water and calcium carbide, shown in equation 5.1.

$$H_2O + CaC_2 \longrightarrow CaO + C_2H_2 \qquad 5.1$$

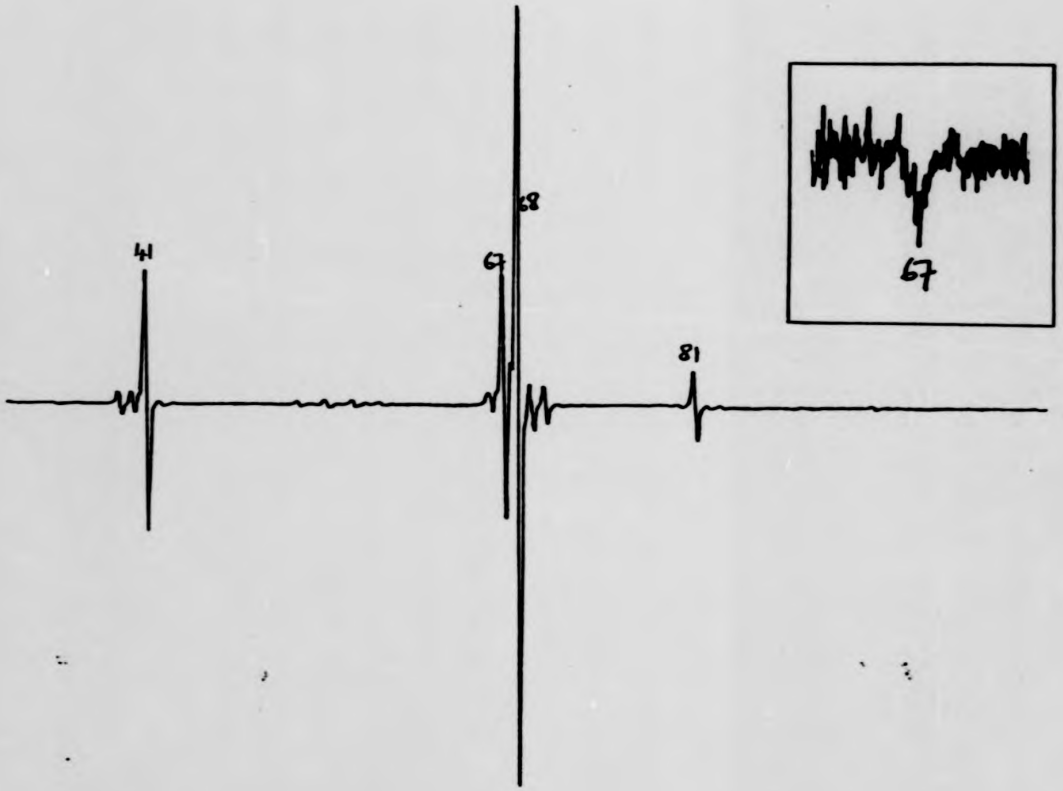


Figure 5.2. Single resonance spectrum of cyclopropyl cyanide.
(Electron energy 13 eV, emission current 100 nA, r.f. field 160 KHz; field modulation; sample pressure 1 x 10^{-5} torr)
Insert:- Double resonance signal for m/z 81 obtained whilst irradiating m/z 67.

5.3 RESULTS AND DISCUSSION

5.3.1 Single Component Systems

The single resonance spectrum of pyrrole consists of three peaks of m/z 41, 67 and 68, with the molecular ion being the dominant peak. The ion of m/z 41 is presumably formed by the loss of C_2H_2 from the pyrrole molecular ion and the ion of m/z 68 is the ^{13}C isotope peak of the molecular ion.

In contrast, the single resonance spectra of allyl cyanide, crotyl cyanide, methacrylonitrile and cyclopropyl cyanide are dominated by an intense peak at m/z 68. This facile protonation is caused by the relatively high proton affinities (190-200 kJ $mole^{-1}$) of the alkyl cyanides.[161] As is the case with the spectrum of pyrrole, a peak at m/z 41 is observed in the spectra of the four cyanides.

The only ion-molecule reaction product observed in the single component systems, other than $(M + H)^+$ ions, occurred at m/z 81 in the spectrum of cyclopropyl cyanide, see Figure 5.2. Double resonance studies indicated that m/z 67 was the sole precursor. This reaction, which has been observed previously,[140] is most likely a $CH_2^{+\cdot}$ transfer from the molecular ion to the neutral cyclopropyl cyanide, as shown in equation 5.2.

$$\underset{}{C_3H_5CN} + \underset{\text{m/z 67}}{C_3H_5CN^{+\cdot}} \longrightarrow \underset{\text{m/z 81}}{(C_3H_5CN\text{-}CH_2)^{+\cdot}} + C_2H_3CN \qquad 5.2$$

This is a significant reaction because it implies that some of the cyclopropyl cyanide molecular ion might exist in a ring-opened form and the reaction would then be as shown in Figure 5.3, although a similar reaction involving the ring closed structure (c) cannot be ruled out.

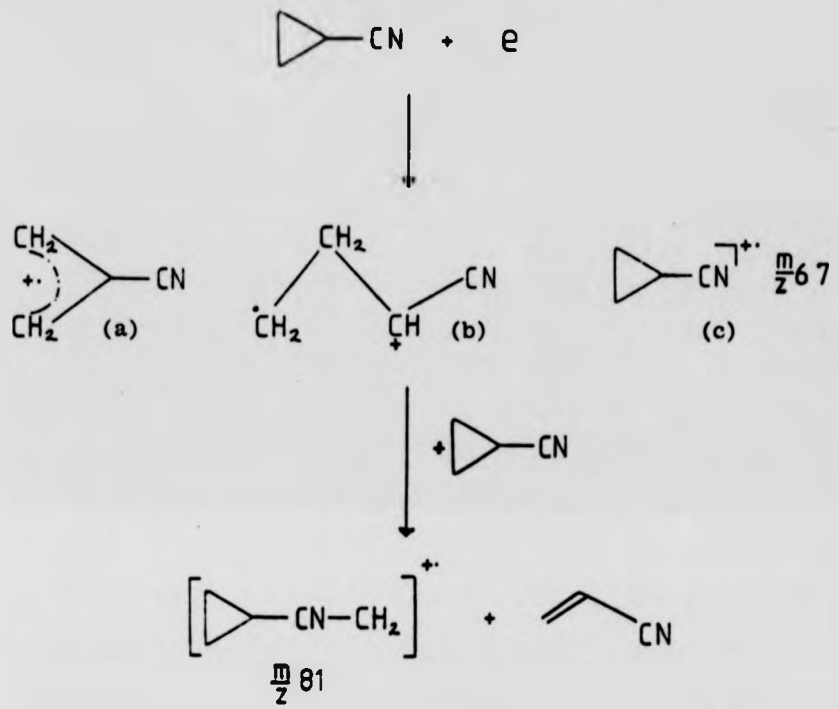


Figure 5.3. Possible structural explanation of the $CH_2^{+\cdot}$ transfer reaction observed in the cyclopropyl cyanide single component system.

5.3.2 *Investigation of a $CH_2^{+\cdot}$ transfer reaction*

In order to make use of the $CH_2^{+\cdot}$ transfer reaction observed in the cyclopropyl cyanide system it is necessary to obtain a neutral molecule, other than cyclopropyl cyanide, to which the cyclopropyl cyanide molecular ion will transfer $CH_2^{+\cdot}$. This neutral substrate can then be used to infer the existence or lack of existence of a similar $C_4H_5N^{+\cdot}$ ion structure in other $C_4H_5N^{+\cdot}$ systems by the presence or lack of a similar $CH_2^{+\cdot}$ transfer. Several neutral substrates were tried and Table 5.3 lists those which were to some degree successful.

Table 5.3. $CH_2^{+\cdot}$ transfer data.

Compound	Molecular Weight	Product Ion m/z	Double Resonance m/z
Cyclopropyl Cyanide	67	81	67
Acetone	58	72	-
Pyridine	79	93	-
Acetonitrile	41	55	68, 67

A possible $CH_2^{+\cdot}$ transfer from the cyclopropyl cyanide molecular ion to acetone and pyridinewas obeserved, although this cannot be verified because the product ion peaks were of too low an intensity to enable double resonance experiments to be performed. In the case of acetonitrile, the intensity of the peak at m/z 55 (possible $CH_2^{+\cdot}$ transfer) was of the same order of magnitude as the peak at m/z 81 (self $CH_2^{+\cdot}$ transfer), but double resonance experiments produced an ambiguous result. A merged signal at m/z 68 and 67 was obtained together with smaller signals at m/z 41 and 42. Ions of m/z 55 were <u>not</u> observed when acetonitrile was examined with the other four C_4H_5N isomers in the I.C.R. instrument.

Therefore the conclusion is that either a small proportion of the $C_4H_5N^{+\cdot}$ molecular ions formed from cyclopropyl cyanide exist in the ring opened form or a small proportion exist in the ring closed form but will react as if in the ring opened form. There is no evidence to suggest that such species exist in the other four C_4H_5N systems.

5.3.3 Reactions in the 1,3-Butadiene / C_4H_5N systems

1,3-Butadiene has been shown to react with furan molecular ions (m/z 68) to give ions of m/z 122 and 107,[162] as shown in equation 5.3.

$$C_4H_4O^{+\cdot} + C_4H_6 \longrightarrow C_8H_{10}O^{+\cdot} \xrightarrow{-CH_3} C_7H_7O^{+} \qquad 5.3$$

m/z 68 m/z 122 m/z 107

Experimental data[162] suggests that a single intermediate is formed which undergoes two competitive methyl expulsions. A mechanism for this reaction is shown in Figure 5.4. Pathways (1) and (2) account for the dominant loss of methyl (80%) and pathway (3) accounts for the minor loss of methyl (20%).

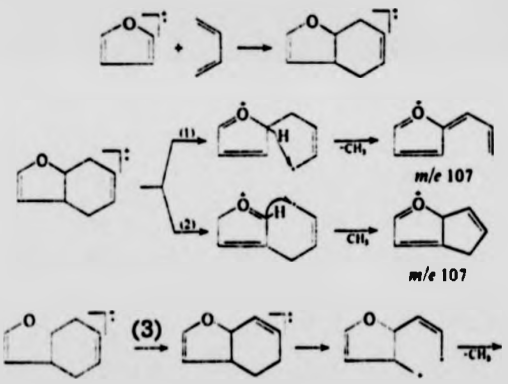


Figure 5.4. Suggested mechanism[162] for the reaction between 1,3-butadiene and ionised furan.

Because pyrrole has a similar structure to that of furan it was thought that an analogous reaction would occur. Ions were observed in the 1,3-butadiene/pyrrole system at m/z 121 and 106, as shown in Figure 5.5. The precursor ion for both products, as indicated by double resonance experiments, was m/z 67, which suggests that the reaction pathway shown in equation 5.4 occurs.

$$C_4H_5N^{+\cdot} + C_4H_6 \longrightarrow C_8H_{11}N^{+\cdot} \xrightarrow{-CH_3^{\cdot}} C_7H_8N^{+} \qquad 5.4$$

m/z 67 m/z 121 m/z 106

The mechanism of this reaction is presumably similar to that proposed for the analogous reaction observed using furan (see Figure 5.4). An

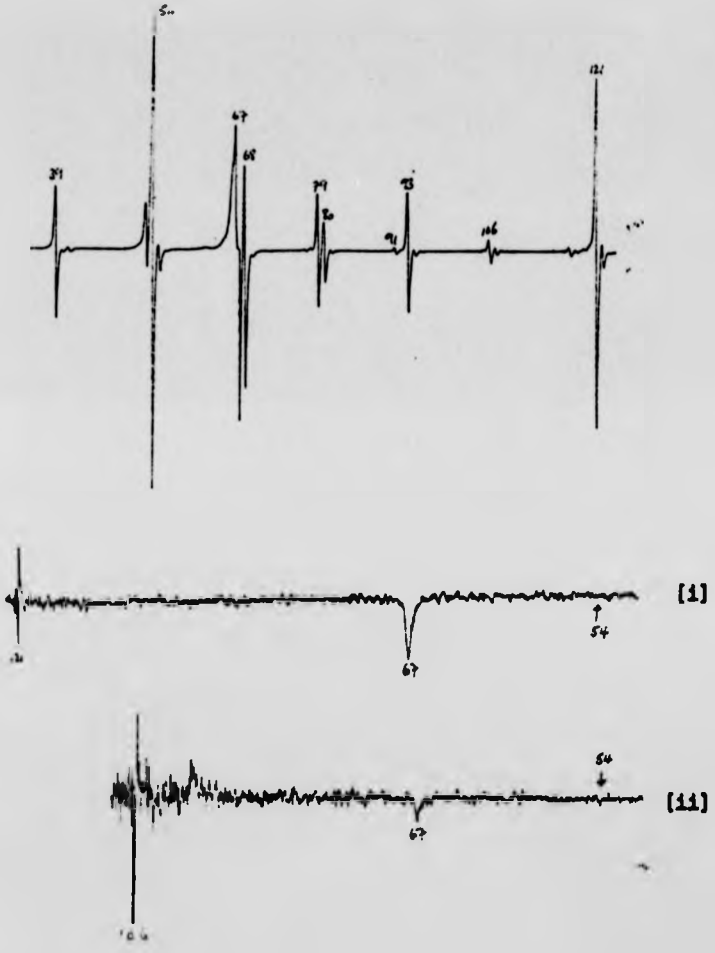


Figure 5.5. Single resonance spectrum of 1,3-butadiene and pyrrole.
(Electron energy 13 eV, emission current 100 nA, r.f. field 120 KHz; field modulation; sample pressure $1 + 1 \times 10^{-5}$ torr).

and

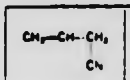
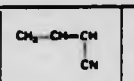
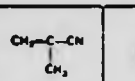
Double resonance spectra for the product ions.

[i] m/z 121 and [ii] m/z 106.

attempt to obtain more information about this mechanism was made by investigating the pyrrole/isoprene system. No clear-cut conclusions could be drawn from this experiment owing to the complexity of the spectrum obtained.

The ion-molecule reaction products observed in the 1,3-butadiene/ C_4H_5N systems are summarised in Table 5.4.

Table 5.4. Product ions observed in the C_4H_5N/1,3-butadiene systems.

 Allylcyanide	 Crotonitrile	 Methacrylonitrile	 Cyclopropyl cyanide	 Pyrrole
[122]	[122]	[122]	[122]	
[121]	[121]	[121]	[121]	121(67)
120(54)	120(54)	120(54)	120(54)	
106(54)	[106]	[106]	[106]	106(67)
-	-	104	104	-

[ ] indicates a low signal intensity

( ) indicates ion precursor, as determined by double resonance.

These results indicate that the reactions observed in the pyrrole/1,3-butadiene system did not occur to any great extent in the other systems. Thus the reaction with 1,3-butadiene allows one to identify $C_4H_5N^{+\cdot}$ ions having the structure of the pyrrole molecular ion.

The major reaction product observed in the systems other than pyrrole involves the 1,3-butadiene molecular ion (m/z 54), as shown in equation 5.5.

$$C_4H_6^{+\cdot} + C_4H_5N \longrightarrow C_8H_{11}N^{+\cdot} \xrightarrow{-H^{\cdot}} C_8H_{10}N \qquad 5.5$$

m/z 54 m/z 121 m/z 120

This reaction is common to all the four cyanides examined. The question arises as to whether the addition of the 1,3-butadiene molecular ion involves the cyanide group or the carbon-carbon double bond functionality in the neutral compounds. In view of the fact that the reaction is common to all the four cyanides examined here and that an addition of 1,3-butadiene to a cyanide group has been observed in synthetic chemistry[163] (see Figure 5.6) it would seem likely that this may be the case in the gas phase.

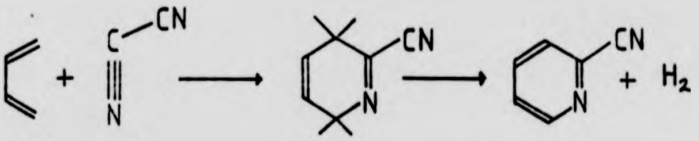


Figure 5.6. Illustration of the production of pyridines using cyanogen and 1,3-butadiene.

This remains speculative and further experiments are needed to test the mechanism of the reaction. These experiments have not been performed because this study is concerned primarily with ionic structure. However, it may be that ionised 1,3-butadiene could be of use for the detection of cyanide groups.

5.3.4 *The search for a characteristic ion-molecule reaction involving ionised methylacrylonitrile*

Having found an ion-molecule reaction that allows the ionised pyrrole molecular ion to be distinguished from other $C_4H_5N^{+\cdot}$ ions, attention was focussed upon ionised methacrylonitrile which, according to C.A. results,[156] was thought to give,[156] at least in part, ions of

a distinct structure. Several substrates were examined in order to find an ion-molecule reaction characteristic of this structure. The substrates were chosen on the basis of two main criteria. Firstly a wide selection of small molecules with different chemical function groups were chosen. Secondly, owing to the high ionisation energies of cyanides, substrates were chosen so as not to diminish the molecular ion intensity by charge transfer. Table 5.5 lists the substrates that were used in this survey together with their ionisation energies.

As can be seen from Table 5.5, no ion-molecule reactions involving $C_4H_5N^{+\cdot}$ (m/z 67) ions were observed. Most of the reactions observed involved either m/z 68 (protonated molecular cyanide ions) or the molecular ions of the substrates, for example m/z 64 in the case of 1,1-difluoroethylene. These reactions will not be discussed more fully because they are not relevant to the aim of this work. They have been discussed elsewhere.[164]

5.4 CONCLUDING REMARKS

A characteristic ion-molecule reaction of the ionised pyrrole molecular ion has been found which allows this ion structure to be differentiated from other ion structures. An ion-molecule reaction, $CH_2^{+\cdot}$ transfer, has been observed involving the cyclopropyl cyanide molecular ion, but it has not been possible to substantiate this reaction unequivocally. This may be due to two factors. Either the $C_4H_5N^{+\cdot}$ structure responsible for the reaction is present as a minor constituent or it is possible that a substrate cannot readily be found to which the $C_4H_5N^{+\cdot}$ ion will transfer $CH_2^{+\cdot}$ in preference to transferring $CH_2^{+\cdot}$ to cyclopropyl cyanide.

Ion-molecule reactions characteristic of $C_4H_5N^{+\cdot}$ structures other than ionised pyrrole could not be found and this illustrates the main

Table 5.5. List of the substrates examined to try and find characteristic ion molecule reactions for the cyanides.

Substrates	I.E.[52] (eV)	$CH_2=CH-\underset{CN}{\underset{|}{CH_2}}$ Allylcyanide	$CH_3-CH=\underset{CN}{\underset{|}{CH}}$ Crotonitrile	$CH_2=\underset{CH_3}{\underset{|}{C}}-CN$ Methacrylonitrile	 Cyclopropyl cyanide
1,1-Difluoroethene	10.3	-	-	X (64)	-
Vinyl Chloride	10.0	-	-	X (68)	-
1-Nitro-propane	10.8	-	-	X (68)	-
1-Butene	9.6	-	-	N	-
Ethyne	11.4	X (68)	X (68)	X (68)	-
Propyne	10.4	X (68)	X (68)	X (68)	-
Tetrafluoroethylene	10.12	X (68)	X (68)	X (68)	-
[a]Acetone	9.7	X (58)	X (58)	X (58)	X (58)
V.M.E.	8.9	N	N	N	N

- : experiment not performed.

N : no reaction products observed.

X : no reaction product giving m/z 67 as a precursor ion.

( ) : the main ion responsible for the reaction products observed.

[a] : see Section 4.5.2.

weakness of the I.C.R. technique for ion structure determination. The main problem encountered throughout this work has been the overwhelming presence of the protonated molecular ions, $C_4H_6N^{+\cdot}$, caused by the high proton affinities of the cyanides.

Apart from a more detailed investigation of the 1,3-butadiene/R-CN reaction (Section 5.3.3) and the $CH_2^{+\cdot}$ transfer reactions (Section 5.3.2) there remains one main area of investigation. Having found a characteristic reaction for ionised pyrrole an application is required to test its use. Sakurai and Jennings[157] have shown that the ions of m/z 67 formed by the loss of CO from 2-hydroxy pyridine, the loss of HCN from 2-amino pyridine and the ionisation of pyrrole exhibit very similar C.A. spectra. Hence, if a suitable solids inlet system was attached to the I.C.R. spectrometer, 1,3-butadiene could be used to probe the structure of the m/z 67 ions formed from 2-hydroxy pyridine and 2-amino pyridine.

CHAPTER 6

THE DETECTION OF GIBBERELLINS

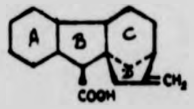
6.1 INTRODUCTION

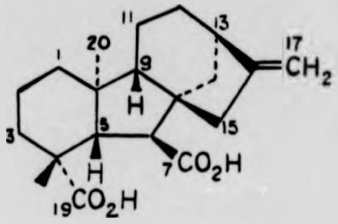
6.1.1 The Gibberellins

The gibberellins comprise a set of diterpenoid compounds which are believed to function as hormones, regulating growth and development in higher plants.[165] They were first discovered[166] early in this century as plant growth promoting (secondary) metabolites of the rice pathogen *Gibberella fujikuroi* and are now known to be present in a large number of green plants. To date fifty-six gibberellins, the structures of which are oxidative variants of the two basic structures shown in Figure 6.1, have been characterised.[167,168]

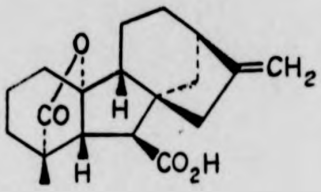
The structures of the various gibberellins of interest in this work are shown in Figure 6.2. For convenience the gibberellins (GAs) will be referred to by their trivial names, GA_1 to GAn.[169]

The hormonal function of the GAs gives their metabolism special importance. The aim of most GA research has been to provide information about the ways in which GAs are synthesised and deactivated and hence to understand the factors which control their levels in plants. The main interest of the plant physiologist involves GA metabolism in the higher forms of plant life. However, practical investigation involving intact plants poses serious problems.[170] Thus studies with cultures and cell-free systems from the fungus *Gibberella fujikuroi* have been used both as a model for work with intact higher plants and to provide more basic information. For example, details are beginning to emerge from such studies of the metabolic pathways of GAs in, *Cucubita maxima* (pumpkin) and *Pisum sativium* (pea).[167] The metabolic pathways, involving gibberellins, that have already been identified in *Gibberella fujikuroi*, cell-free plant preparations and intact plants have recently been reviewed.[167,170]



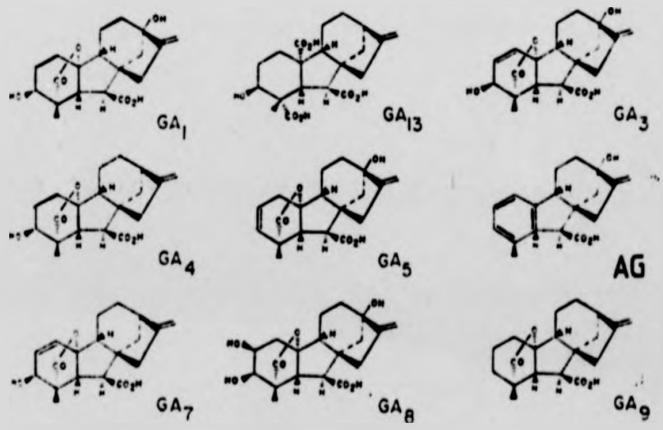


C_{20}-gibberellin



C_{19}-gibberellin

Figure 6.1. Basic gibberellin structures.



AG = Allogibberic Acid

Figure 6.2. Structures of the gibberellins used in this work.

6.1.2 *Methods of Gibberellin Analysis*

In order to follow the metabolic pathways of the GAs and to identify the various products, sensitive and selective analytical techniques are required. Techniques such as mass spectrometry, infra-red and nuclear magnetic resonance, all provide a definitive identification, with mass spectrometry being the most sensitive. The simpler techniques such as bioassay, thin layer chromatography and gas liquid chromatography are not specific in themselves although identification becomes more likely the greater the number of chromatographic systems being used. Thus gas chromatography/mass spectrometry (G.C./M.S.) which provides both the sensitivity and specificity that is lacking in the various other techniques has become established as a "procedural cornerstone in the rigorous analysis of gibberellins".[171]

Before proceeding to describe the aim of the present work, it is instructive to consider the use of G.C./M.S. for GA analysis. The advantages of the technique can be summarised as follows:-

1. It is a sensitive technique.
2. It can be applied to mixtures.
3. It allows conclusive identification of the GA, provided reference spectra are available.
4. It can be used for semi-quantitative analysis, if calibration standards are available.

Apart from the high capital cost, the main disadvantage of this technique, and indeed of other techniques, lies in the practical problems of sample preparation which make the analysis a lengthy and hence costly procedure. To understand this more clearly the various stages involved will be considered briefly.

The G.C./M.S. analysis of GAs consists of five basic stages, which have been discussed in detail in reference 172. These are:-

1. Extraction/Purification	SAMPLE PREPARATION
2. Derivatisation	
3. Gas Chromatography	G.C./M.S.
4. Mass Spectrometry	
5. Data Analysis	

Stages 1, shown in more detail in Figure 6.3, and 2 are the most lengthy and costly. Since GAs are present in micro-gram quantities, care must be taken not to introduce contaminants[171] from undistilled solvents, teflon, aluminium foil, grease or detergents. If the extracted fractions are not concentrated enough then further purification procedures, such as thin layer chromatography, partition chromatography or high pressure liquid chromatography may be used.[172] Gibberellins, being carboxylic acids, decompose under the usual gas chromatographic conditions. Hence it is necessary to form volatile, thermally stable derivatives. Derivatisation procedures generally used[172] involve the methylation of carboxylic acid groups and silylation of hydroxyl groups. The use of capillary columns[173] instead of packed columns has greatly increased the quality and simplified the technique of gas chromatography. Nevertheless a typical G.C. experiment will still take an hour.

6.1.3 Aim of this Work

In recent years a great deal of effort has been expended upon the development of various techniques known as Mass Spectrometry/Mass Spectrometry (MS/MS).[174] These methods are aimed at removing the need for the separation of mixtures prior to mass spectrometric analysis of the component(s) of interest. In order to achieve this a mass

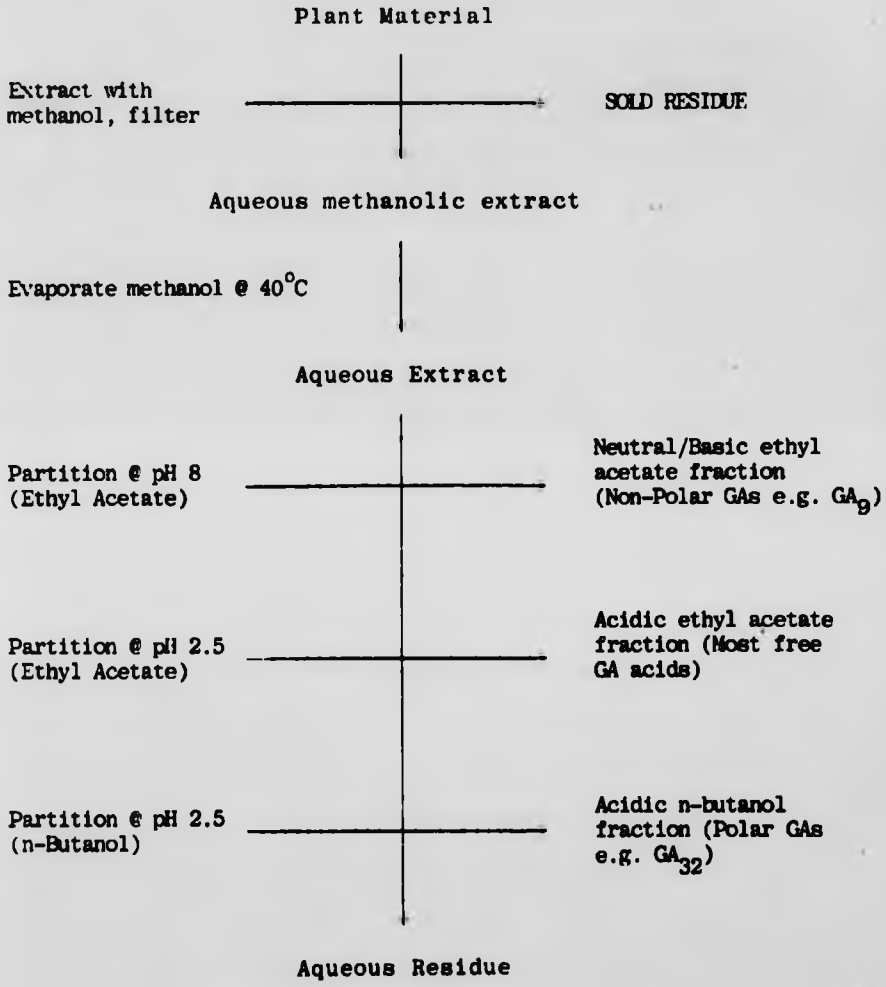


Figure 6.3. Simplified scheme for the extraction of gibberellins. (Adapted from reference 172)

spectrometer is used to perform the two roles of separation and detection.

The aim of this work is to apply the philosophy of MS/MS to the analysis of gibberellins. Making the gas chromatograph redundant eliminates, in this case, the need for derivatisation and allows a crude plant extract to be used thus reducing the time, cost and effort of analysis.

One MS/MS technique employed when using a Nier-Johnson mass spectrometer, such as the MS50, utilises the techniques of collision induced decomposition and B/E linked scanning as described in Chapter 2.[175] The basic experimental approach used in this work, see Figure 6.4, involves three stages of separation. The crude sample is admitted into the ion source on the end of a probe. The first stage of separation involves desorption off the probe. This is controlled rather crudely by the source/reagent gas temperature. Secondly, a chemical separation is effected. Chemical ionisation is used to produce a structurally significant high mass ion of the component of interest by means of a selective gas phase ion-molecule reaction. Finally, this ion is selected using the magnet. Detection is accomplished by collecting the collision induced decomposition products (Section 2.4.3) of the selected ion using a B/E linked scan.[71] The spectrum obtained is dependent upon the structure of the precursor ion (Section 4.2.2 or reference 122). By comparison with other reference B/E spectra, this ion and hence the neutral compound from which this ion is derived can be identified.

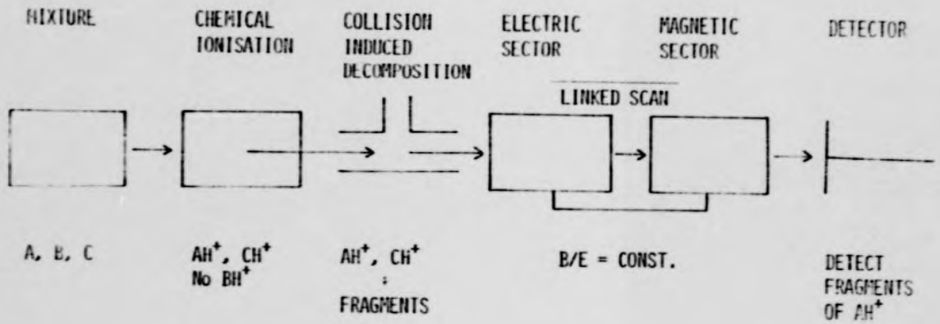


Figure 6.4. Experimental method.

6.2 EXPERIMENTAL

Initial exploratory experiments were performed in the mass spectrometry laboratories belonging to Shell Research Limited, Sittingbourne, Kent, using an MS30 (Kratos Analytical Instruments Limited) double focussing mass spectrometer interfaced to a Multi-Spec (Vacuum Generators Limited) data system. The MS30 is a double beam mass spectrometer which allows accurate mass measurements to be made with ease, using either electron impact or chemical ionisation techniques. Adjacent sources are used to produce two ion beams which pass simultaneously through the same analyser system and arrive at independent collectors. The compound(s) to be examined are ionised in the first source, whilst a reference compound which produces ions of known mass(es) is admitted to the second ion source. In this case perfluorokerosene (PFK) was employed as the reference compound.[176] By means of a computer, the arrival time at collector 1 of the ions formed in source 1, is compared with the arrival time at collector 2 of the reference ions. Dedicated electron impact or chemical ionisation sources (Beam 1) and an electron impact source (Beam 2), operating at 4 kV (600 daltons mass range) were used. Electron impact spectra were obtained using an electron energy of 70 eV and a trap current of 100 μA. A trap current of 500 μA and electron energies of 150 eV and 200 eV were used when operating in the positive and negative chemical ionisation modes respectively. Solid samples were admitted to the ion source using a heated direct insertion probe with a pyrophylite tip. Reagent gases were admitted to the ion source either from a gas cylinder or as a vapour from a reservoir via a manual leak valve. The source pressure was monitored indirectly using an ion gauge mounted between the source and diffusion pump of the source pumping line. Source temperatures of 150°C (Negative ion mode) and 100°C (Positive ion mode)

were employed and the probe was heated rapidly to 250-300°C.

Further experiments involving metastable scanning and accurate mass measurements by peak matching[177] were performed using an MS50 (Kratos Analytical Instruments Limited) double focussing mass spectrometer. A chemical ionisation source (Mark I) was operated at 6 kV accelerating voltage, using an electron energy of 200 eV and a trap current of 500 μA. A Hall probe and a Hirst FM 70A Fluxmaster were used to monitor the magnetic field strength. B/E linked scans were obtained using a home-built electronic control unit, which monitors the electric sector reference voltage (E_{ref}), the accelerating reference voltage (V_{ref}) and the value of the magnetic field (B) and also controls the scanning of the electric sector voltage (E) and the magnetic field (B) in a fixed ratio. This ratio and the starting point of the B/E linked scan is defined by the ratio B_o/E_o where B_o is the value of the magnetic field at the centroid of the peak of interest and E_o is the normal value of the electric sector voltage. All B/E linked scan spectra were recorded on ultra-violet chart paper and the magnet was cycled prior to obtaining each spectrum. The source slit aperture was fully open (0.25 mm) and the collector slit was closed to give 90% transmission. A collision chamber, situated between the adjustable source slits and the z-restrictor,[178] was used in conjunction with the B/E linked scan to obtain collision induced decomposition spectra. Experiments were performed using Helium as a collision gas. Solid samples were introduced into the ion source using an unheated D.C.I. probe as described previously (Section 2.2.6). The reagent gas, ammonia, was admitted to the ion source via a one litre heated glass reservoir using a manual leak valve, with the source pressure being monitored by an ion gauge remotely situated on the source diffusion pumping line.

For the mixture analysis work (Section 6.3.5), standard grass extract solutions were obtained by crushing grass in a blender with some methanol and filtering off the residue. The filtrate was then rotary evaporated and the residue thus obtained was weighed and re-dissolved in methanol in known amounts, for example 1 µg per µl.

Gibberellin samples were kindly supplied by Professor J. MacMillan, Bristol University, with the exception of GA_3 which was obtained from the Aldrich Chemical Company. The reagent gases used were obtained from Mathesons, British Oxygen Company and Air Products.

6.3 RESULTS AND DISCUSSION

6.3.1 Introduction

The development of an MS/MS technique for the analysis of GAs involves three basic stages. These are:-

1. The development of the correct sample handling conditions and ionisation method to facilitate the production of a high concentration of a structurally significant ion of the gibberellin of interest (Section 6.3.2 and 6.3.3).
2. The building up of a library of B/E reference spectra of the ions of interest (Section 6.3.4).
3. The application of stages 1 and 2 to detect the presence of gibberellins in mixtures and the investigation of the scope and limitations of the technique (Section 6.3.5)

In order to clarify the following sections and to place the following work in the context of this thesis, it is necessary to make a few general points. The first stage involves the use of chemical ionisation (Section 2.2.2) to find an ion-molecule reaction that will

selectively ionise the gibberellins present in the ion source in a mixture. The philosophy behind the choice of reaction will be discussed later when the central role played by the selected ion-molecule reaction in this method of mixture analysis will become apparent. During the preliminary C.I. studies involving GA_3, a sample handling problem became apparent. Further investigations led to the conclusion that GA_3 pyrolysed readily. Because this fact is in conflict with previously reported data[179] and because it led to a change in the method of sample handling used for the preceding work the pyrolysis was investigated in some depth. Although these studies were conducted at the same time as stages 1 and 2, for the sake of clarity the pyrolysis of GA_3 has been discussed in a separate section (Section 6.3.3). Experiments peformed during stage 2, to investigate the effect of a collision gas in the first field free region of the mass spectrometer, revealed little effect upon the B/E linked scan spectra obtained (see Section 6.3.4). It was concluded that owing to the conditions prevalent, collision induced decompositions involving the reagent gas were already occurring. Therefore, all the B/E linked scan spectra presented in this chapter contain, at least in part, peaks due to collision induced decomposition, although no collision gas has been specifically added. In the final stages of this work, time did not permit the application of this method to the detection of GAs in naturally occurring mixtures. However, results are reported, Section 6.3.5, concerning the detection of gibberellins in spiked crude grass extracts, allowing some conclusions to be made about the applicability of the technique.

6.3.2 *Chemical Ionisation Studies of the Gibberellins*

6.3.2.1. Rationale

Electron impact ionisation is ideally suited to the analysis of GAs by G.C./M.S., providing structurally diagnostic spectra of the individual gibberellins as they elute into the ion source. However, when a mixture is present in the ion source then the multitude of ionic species formed serve to obscure any structural information present. In such cases it becomes imperative to simplify the spectra obtained by concentrating the ion current in as few different ions as possible, ideally, in one type of ion per component. Chemical ionisation (Section 2.2.2) represents one approach towards this goal. Furthermore, chemical ionisation allows a suitable choice of reagent ion to be made that discriminates (positively) in favour of a particular component present in a matrix, so increasing the sensitivity and selectivity of the technique as a whole. The factors to be borne in mind when choosing a suitable reagent ion can be summarised as follows:-

(i) Molecular Weight/Structural Information

The ion should react with the gibberellin of interest to produce a high mass ion, ideally a molecular ion or simple molecular adduct ion. This preserves molecular weight information and maximises the amount of structural information present in one ion.

(ii) Sensitivity

The reaction should produce as few fragment ions as possible thereby concentrating the total ion intensity of the compound of interest and enhancing the sensitivity of detection.

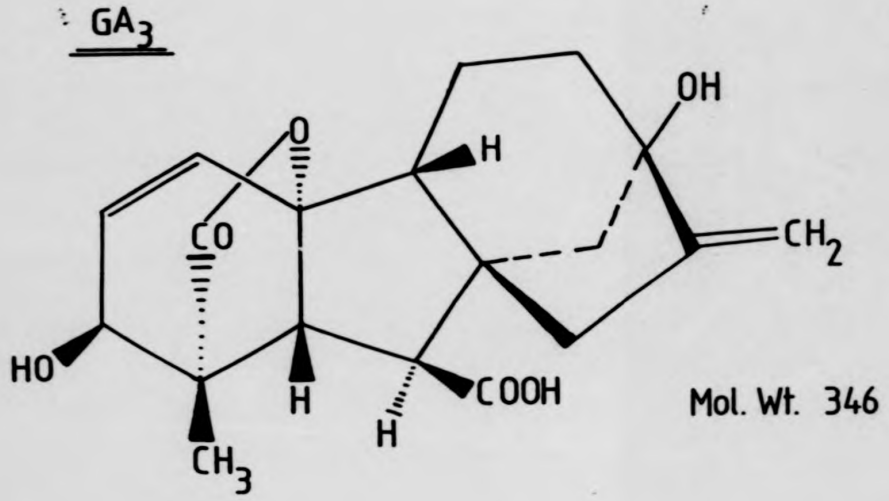


Figure 6.5. The structure of Gibberellic Acid (GA_3) - a ring A unsaturated C_{19} gibberellin.

(iii) Selectivity

The ion should react with as little as possible, other than the gibberellin.

(iv) Convenience

The ion should be produced in a simple manner from a gas or mixture of gases that is non-toxic, instrumentally clean, cheap and generally available.

As far as can be ascertained, no chemical ionisation studies involving gibberellins have been reported. Gibberellic acid (GA_3), Figure 6.5, is the most generally available gibberellin and hence, was chosen to be studied first.

6.3.2.2. Electron Impact Ionisation

By way of an introduction to the chemical ionisation results and in order to provide necessary background information to the ensuing sections, the behaviour of GA_3 under electron impact conditions will first be considered. The fragmentation of the GA_3 molecular cation has been considered in some detail.[179] Figure 6.6 shows the electron impact spectrum of GA_3 obtained using a source temperature of 250°C and a probe temperature of 200°C.

Note that m/z 284 and m/z 136 constitute the base peaks of this spectrum and that the relative intensities of the peaks of m/z greater than 284 are less than 20% of the base peak with the molecular ion being only 5%. The fragmentation assignments shown in Figure 6 were based upon accurate mass measurements. Note that the loss of H_2O and CO_2 from the molecular ion, to give m/z 284, which then fragments in several different ways, appears to be the major fragmentation pathway. The fragmentation

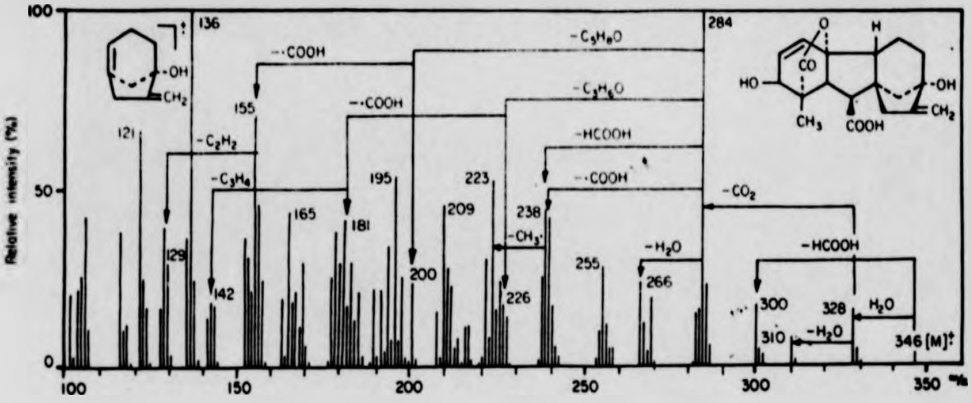
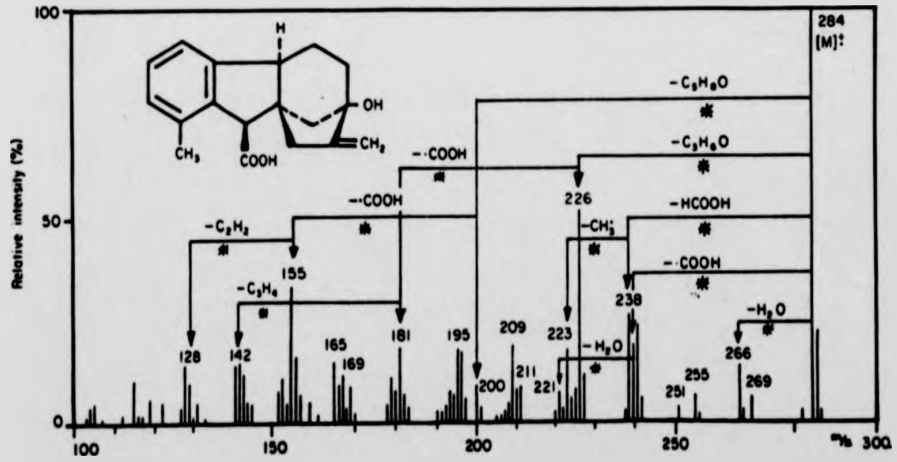


Figure 6.6. A 70 eV mass spectrum of gibberellin A_3.[179]



[* - indicates that the decomposition has been assigned by the observation of a metastable transition]

Figure 6.7. A 70 eV mass spectrum of allogibberic acid.[179]

pattern below m/z 284 was found to resemble quite closely that of allogibberic acid, shown in Figure 6.7.[179]

Therefore, it was concluded[179] that the molecular ion of GA_3 loses water and carbon dioxide to produce an ion of structure similar to that of the molecular ion of allogibberic acid.

6.3.2.3. Positive Chemical Ionisation

Preliminary investigations involved the common positive chemical reagent gases, methane, isobutane and ammonia. The simplicity of the GA_3 spectra obtained under chemical ionisation conditions is illustrated in Figures 6.8 and 6.9. Notice the lack of low mass peaks, m/z less than 200 daltons, observed in the methane chemical ionisation spectrum compared with the electron impact spectrum (Figure 6.8). This simplicity, which can be attributed to the low energetics of the technique of chemical ionisation (Section 2.2.2) is mirrored in the isobutane spectrum of GA_3 and is even more pronounced when ammonia is used as the reagent gas (Figure 6.9).

Figure 6.9 allows one to make a comparison of the high mass peaks (m/z greater than 200 daltons) observed in the electron impact, methane chemical ionisation, isobutane chemical ionisation and ammonia chemical ionisation spectra of GA_3. As expected, the intensity of the protonated molecular ion (m/z 347) increases and the degree of fragmentation observed decreases when isobutane is used in place of methane (see Section 2.2.3). The GA_3/ammonia spectrum, which consists of essentially two peaks of m/z 364 and 302, is thus the simplest spectrum. The formation of an adduct ion $(GA_3 + NH_4)^+$, at m/z 364 is not surprising, bearing in mind the number of oxygen containing functional groups present in the GA_3 molecule (see Section 2.2.3).

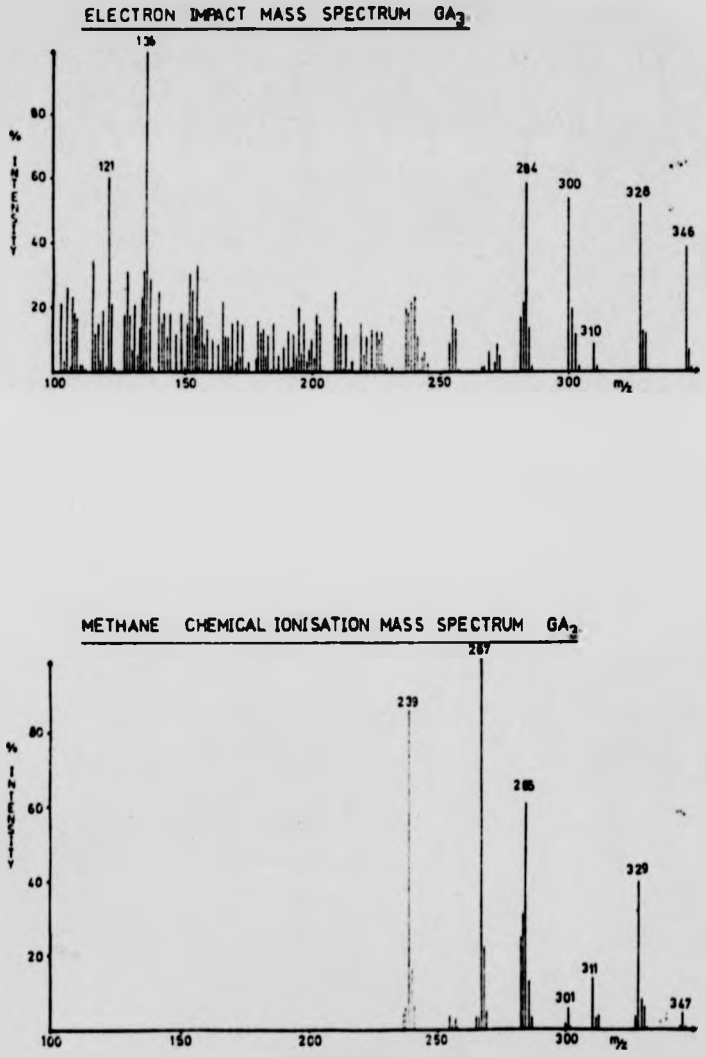


Figure 6.8. Comparison of the methane C.I. and E.I. spectra of GA_3.

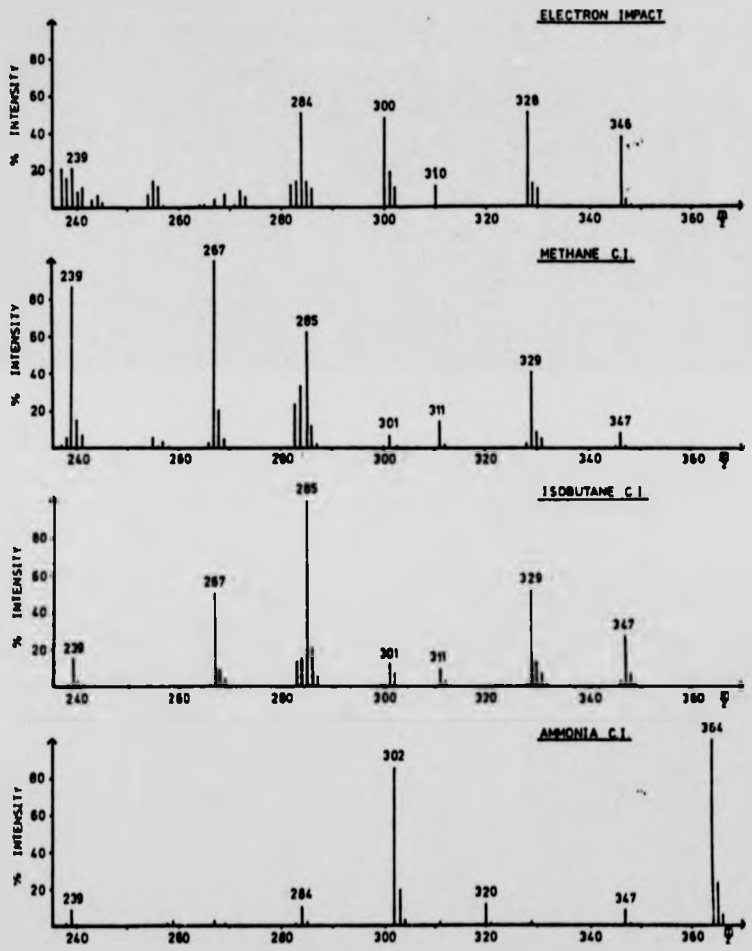


Figure 6.9. Comparison of the peaks observed in the E.I. and C.I. spectra of GA_3 for m/z > 200 daltons.

Table 6.1. Accurate mass data, elemental compositions and fragmentation assignments for the six major ions observed in the methane and isobutane chemical ionisation spectra of GA_3.

OBSERVED MASS		ELEMENTAL COMPOSITION	ERROR (p.p.m.)		MOST PROBABLE ASSIGNMENT
CH_4/GA_3	C_4H_{10}/GA_3		CH_4/GA_3	C_4H_{10}/GA_3	
374.1479	347.1517	$C_{19}H_{23}O_6$	4	- 7	$(GA_3 + H)^+$
329.1327	329.1306	$C_{19}H_{21}O_5$	18	25	$[(GA_3 + H) - H_2O]^+$
311.1252	311.1295	$C_{19}H_{19}O_4$	10	- 4	$[(GA_3 + H) - 2H_2O]^+$
285.1376	285.1385	$C_{18}H_{21}O_3$	40	27	$[(GA_3 + H) - CO_2, H_2O]^+$
267.1345	267.1320	$C_{18}H_{19}O_2$	14	24	$[(GA_3 + H) - CO_2, 2H_2O]^+$
239.1339	239.1414	$C_{17}H_{19}O_1$	41	9	$[(GA_3 + H) - CO_2, H_2O, HCOOH]^+$

Accurate mass measurements, shown in Table 6.1, of six major high mass peaks, observed in the GA_3/methane and GA_3/isobutane spectra, indicate that the ions observed under chemical ionisation conditions may be formed by losses of neutral molecule and radical species similar to those observed in the electron impact spectrum of GA_3[179] (see Figure 6.7). The accurate mass of the ion of nominal m/z 302, observed in the GA_3/ammonia chemical ionisation spectrum, was determined to be 302.1761 ± 0.0006 (2 ppm). On the basis of this result, which was obtained by peak matching with the carbon-13 isotope peak of protonated coronene, an elemental composition of $C_{18}H_{24}O_3N_1$, the accurate mass of which deviates from the measured mass by 1.6 ppm, has been assigned to this ion. The formation of this ion could be due to the loss of the elements of water and carbon dioxide from the adduct ion $(GA_3 + NH_4)^+$. However on the basis of other results, see Section 6.3.3, the formation of this ion has been attributed to the production of an ammonium adduct ion of a pyrolysis product of GA_3, that is $(GA_3 - H_2O, CO_2 + NH_4)^+$.

6.3.2.4. Ammonia Chemical Ionisation

An ammonia chemical ionisation survey involving GA_1, GA_4, GA_5, GA_7, GA_8, GA_9, GA_{13} and allogibberic acid, the structures of which are shown in Figure 6.2, was undertaken. With the exception of GA_{13}, all these gibberellins exhibited similar spectra, consisting of an intense peak due to the $(GA + NH_4)^+$ ion and very little fragmentation. Indeed the second most intense peak, in all these cases, was due to the carbon-13 isotope peak of the ammonium adduct ion, which is of the order of 20% of the intensity of the $(GA + NH_4)^+$ peak.

The ammonia chemical ionisation spectrum of GA_{13} (molecular weight 378) exhibits an intense peak of m/z 378 and very little else. The formation of this ion, as opposed to an ammonium/molecular adduct ion of m/z 396 is discussed in Section 6.3.3.

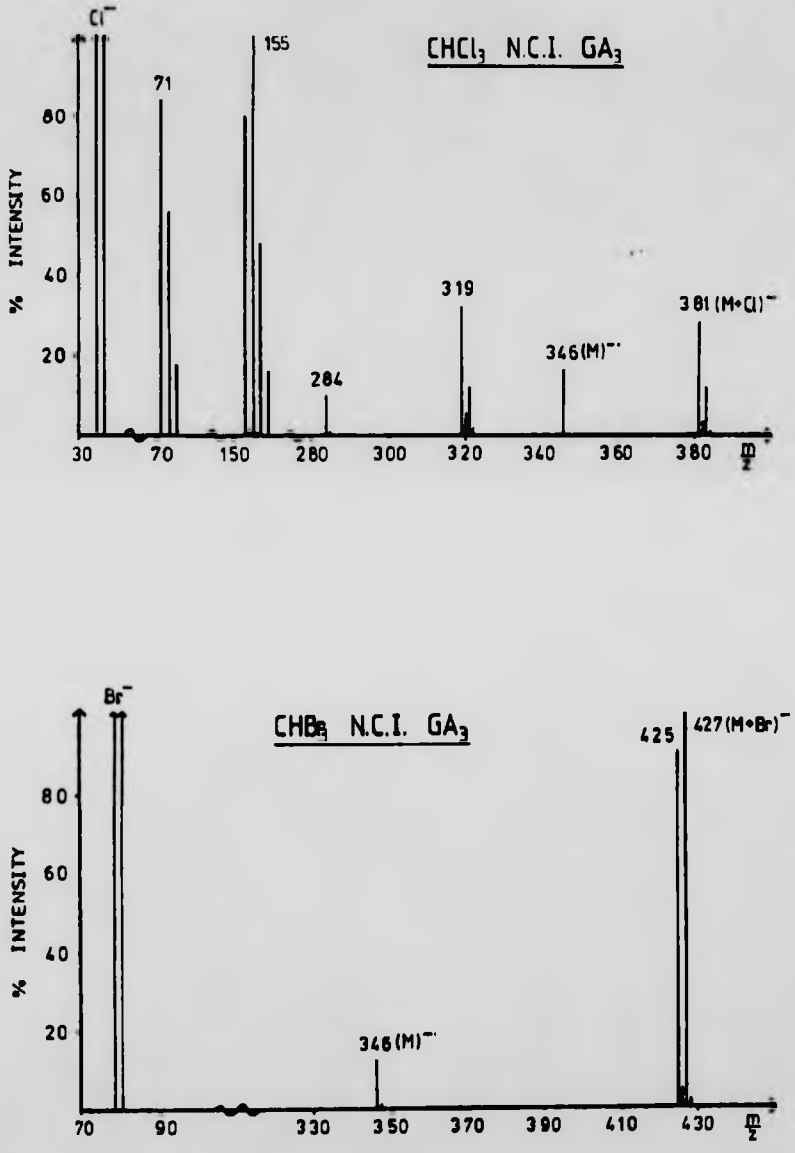


Figure 6.10. Negative chemical ionisation spectra of GA_3
(a) Chloride attachment.
(b) Bromide attachment.

Thus the ammonium ion reacts with a variety of GAs in a simple manner, producing high mass ammonium adduct ions with little fragmentation. It selectively reacts with high proton affinity compounds (Section 2.2.3) and can be produced from ammonia, a commonly used reagent gas. For these reasons it has been chosen as the reagent ion to be used in this work.

6.3.2.5. Negative Chemical Ionisation

The possibility of using negative reagent ions was considered, since they have been shown to be more sensitive than positive reagent ions for some compounds containing electronegative elements and thus might be more chemically selective for such compounds.[180] Figure 6.10 illustrates the spectra obtained using (a) GA_3/chloroform (chloride ion attachment) and (b) GA_3/bromoform (bromide ion attachment). Although the spectra obtained were relatively simple it was felt that, in this case, the use of negative reagent ions offered no great advantage over the ammonium reagent ion. Another factor against the use of negative ions, for this type of work, is the problem encountered in detecting the fragments produced from negative ions by collision induced decomposition. Such fragments tend to be either neutral molecules or positive ions[181] and hence this makes the use of the linked scan insensitive or complicated.

6.3.3 The Pyrolysis of GA_3 and GA_{13}

In retrospect, GA_3 was a difficult sample to handle owing to pyrolysis problems. Several observations led to this conclusion and to a further investigation of this particular case.

Initially, it proved difficult to obtain reproducible chemical ionisation spectra. Ions of m/z greater than 285, observed in the methane and isobutane spectra of GA_3, or greater than m/z 302 in the case of ammonia, were of a transient nature or were frequently non-existent. The only ion that could be obtained with any degree of

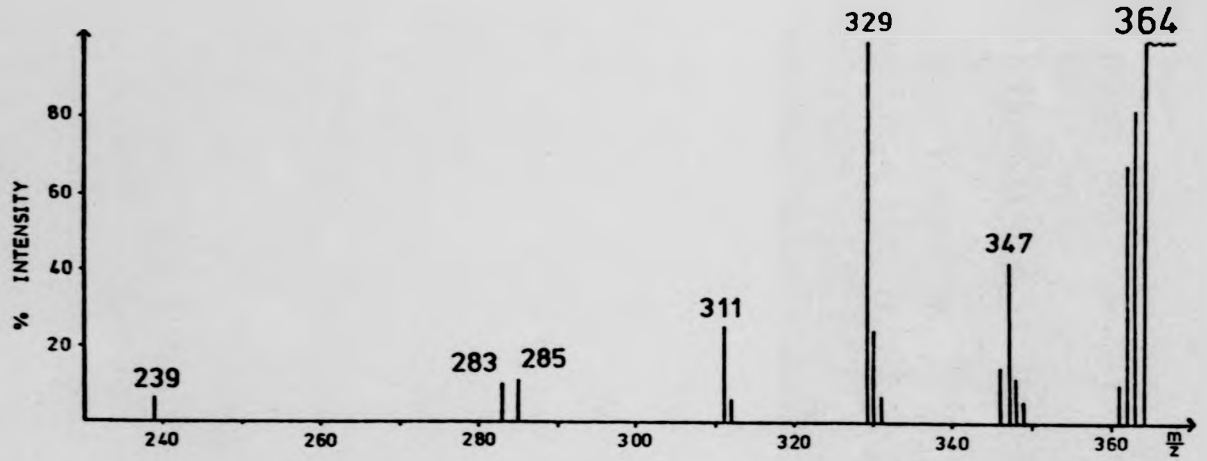


Figure 6.11. A collision induced decomposition spectrum of m/z 364 $(GA_3 + NH_4)^+$.

certainty and reproducibility was that of m/z 285 or m/z 302 in the case of ammonia, which was generally the base peak of the chemical ionisation spectrum. Thus, the results of the chemical ionisation studies of GA_3, Figures 6.8 and 6.9, together with the accurate mass measurements, Table 6.1, appeared to be in agreement with the reported electron impact data in that they indicate that the loss of water and carbon dioxide is a major fragmentation route for GA_3 (see Section 6.3.2).

At this stage it was observed that lowering the source temperature from 250°C (Figure 6.6) to 150°C (Figure 6.8) led to an increase in the relative intensities of the high mass (m/z greater than 284) peaks in the electron impact spectra of GA_3. Therefore, in order to obtain reproducible spectra, an extended probe, shown in Figure 2.2(b) and described in Section 2.2.6, was built. The probe is heated indirectly by the source block and the reagent gas. This method of producing a low temperature vapour inside the ion chamber gave more consistent spectra than the earlier method, which utilised rapid localised heating.

Whilst using this probe to study the GA_3/ammonia system, several experiments suggested that the ion of m/z 302 was not in fact wholly produced by fragmentation. Firstly, it was noted that the intensity of the ion of m/z 364 decayed at a much faster rate than that of m/z 302. Secondly, a probe withdrawal experiment, whereby the intensities of the ions of m/z 364 and 302 were monitored whilst the probe was withdrawn and then re-inserted, gave the following result. When the probe was re-inserted, the intensity of the ion of m/z 364 never regained its value prior to withdrawal whereas the intensity of the ion of m/z 302 surpassed its original value. Thirdly, examination of the B/E linked scan of the ion of m/z 364, shown in Figure 6.11, revealed the absence of a peak of m/z 302, indicating that the fragmentation of m/z 364 to m/z 302 does not occur. In view of the relative intensities of the

fragment ions observed in the B/E linked scan of m/z 364 compared with their relative intensities in the GA_3/ammonia chemical ionisation spectrum (Figure 6.9), it would appear that the major mechanism contributing to the formation of the m/z 302 ion is not that of fragmentation from m/z 364. This leaves two possibilities both of which involve the presence of a second compound either present as an impurity in the GA_3 sample or, more likely in view of the preceding evidence, formed as a pyrolysis product. In view of the electron impact data, it would seem reasonable to assume that this compound is, or has a structure similar to, allogibberic acid.

The presence of allogibberic acid as a major impurity can be ruled out. The melting point of allogibberic acid was determined to be 196-197°C, some 25°C lower than the value of 223-224°C determined for GA_3 (Expected value for GA_3 227°C.[182]). Hence any allogibberic acid present in the GA_3 sample would be expected to volatilise first. Finally, GA_3 decomposed upon melting, supporting the pyrolysis hypothesis.

Therefore, there seems little doubt that GA_3 pyrolyses readily by the loss of water and carbon dioxide. The question arises as to the structure of the pyrolysis product. Figure 6.12 shows the B/E linked scan spectra of the ion of m/z 302 formed using (a) GA_3/ammonia and (b) allogibberic acid/ammonia. With the exception of the peak at m/z 226 in Figure 6.12(b), these spectra are similar, implying that the structure of these ions, formed from two different sources, is similar. (It should be noted that the electron impact spectrum of allogibberic acid, Figure 6.7, also shows a more intense peak of m/z 226 compared with the electron impact spectrum of GA_3, Figure 6.6.) Hence, it is likely that the neutral structures giving rise to these two ions are also similar and it may be concluded that GA_3 pyrolyses by the loss of the elements of water and carbon dioxide from the A ring

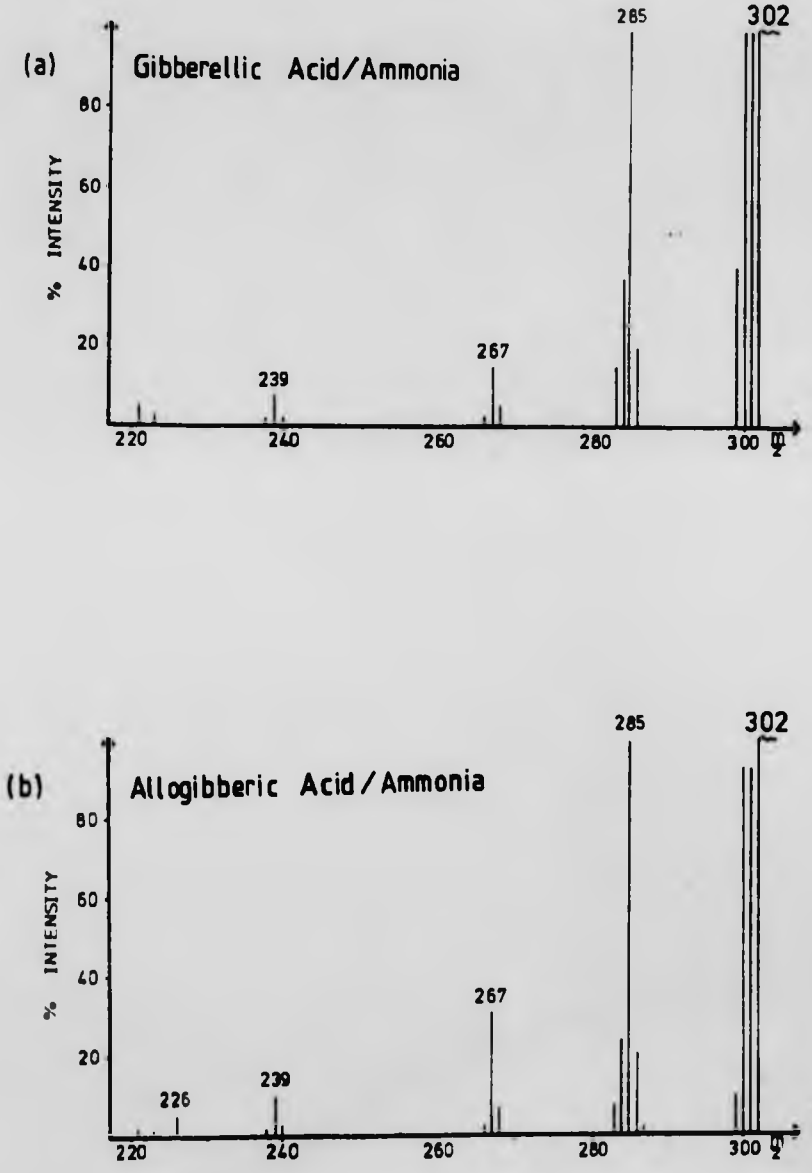


Figure 6.12. Collision induced decomposition spectra of m/z 302 generated from two different sources.

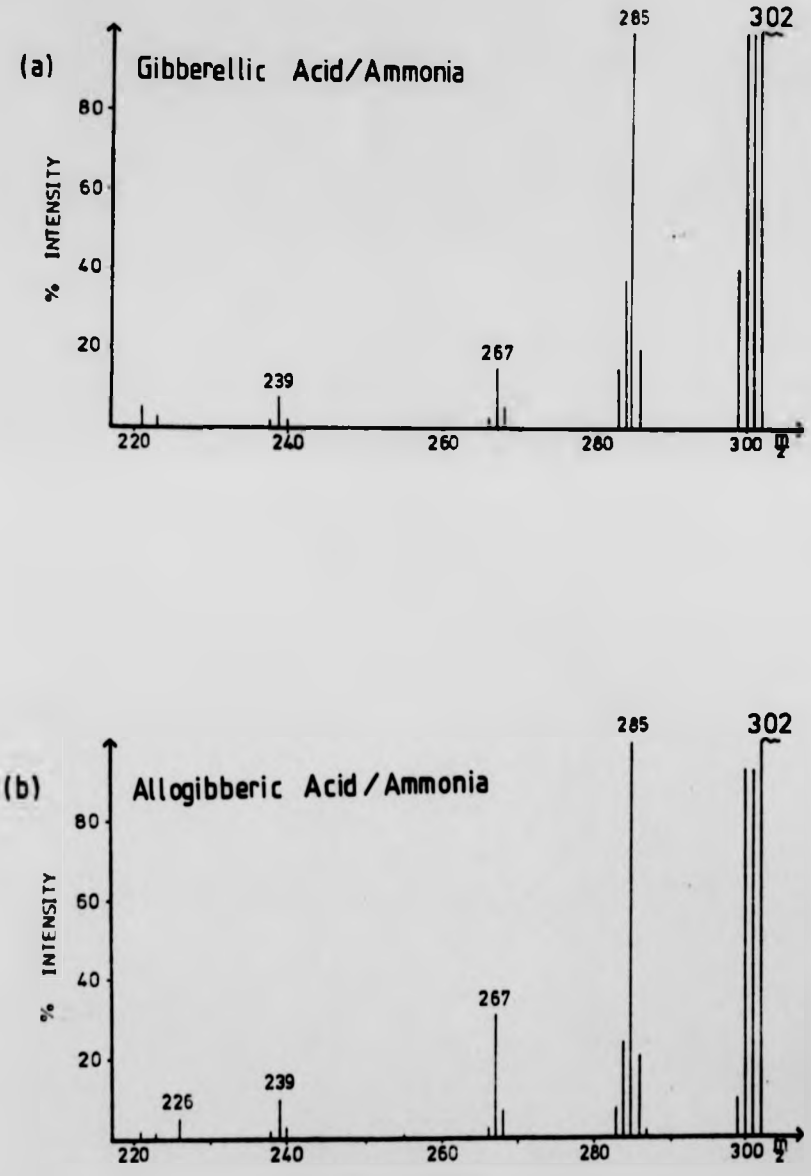


Figure 6.12. Collision induced decomposition spectra of m/z 302 generated from two different sources.

to give an aromatic ring A structure similar to allogibberic acid or epi-allogibberic acid. (Figure 6.13).

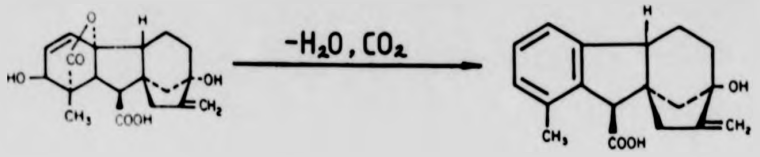


Figure 6.13. Most likely route for the pyrolysis of GA_3.

At first sight this appears to contradict the previously published electron impact results.[179] (Section 6.3.2) However, examination of this data reveals that the decompositions in the allogibberic acid spectrum, Figure 6.7, were assigned *directly* by the observation of metastable decompositions (see Section 2.4.2) whereas fragmentations in the GA_3 spectrum, Figure 6.6, were assigned only *indirectly* by accurate mass measurement. This latter method has been shown to be unreliable if pyrolysis occurs.

The only other gibberellin examined in this work which suffered from major pyrolysis problems was GA_{13} (molecular weight 378). The ammonia chemical ionisation spectrum of GA_{13} is dominated by an intense peak of m/z 378, which is presumably formed by the pyrolytic loss of water from GA_{13} followed by the formation of an ammonium adduct ion.

6.3.4 *Collision Induced Decomposition Spectra (B/E Linked Scans)*

Having decided upon the use of ammonia to effect ionisation, the B/E linked scan spectra of the appropriate gibberellin/ammonium adduct ions were obtained. These spectra are reproduced in Figures 6.14 and 6.15 and summarised in Table 6.2. Prior to a discussion of the analytical and structural implications of these results, the conditions under which they were obtained will be considered.

Experiments were performed to investigate the effect upon the B/E (linked scan) spectrum of the m/z 364, $(GA_3 + NH_4)^+$, ion of the introduction of collision gas, helium, in the first field free region of the mass spectrometer. Reduction of the transmission of the main beam by either 50% or 90% produced no radical changes in the fragmentation pattern observed. As the collision gas pressure increased, the intensity of the peak of m/z 347 showed a slight relative decrease and the intensity of the peak of m/z 283 increased slightly relative to that at m/z 285. The absolute intensity of the peaks observed decreased with increasing pressure of collision gas. The observations enable one to draw the following conclusions. Due to the high pressure of reagent gas in the ion source and the construction of the collision chamber close to the ion source, [178] collision induced decompositions involving reagent gas molecules were occurring. Hence the presence of additional collision gas serves only to reduce the sensitivity of the technique. Although it was not therefore possible to delineate the metastable and collision induced parts of the spectrum reproducible results could be obtained by always working at the same constant reagent gas pressure.

The B/E spectra of the various $(GA + NH_4)^+$ ions reflect the different structures of the gibberellins examined (see Section 4.2.2 and/or reference 122). Comparison of the spectra, Figures 6.14 and

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Table 6.2. Summary of the various fragment ions observed in the B/E linked scans of nine gibberellin/ammonium adduct ions.

GIBBERELLIN			GA_3	GA_7	GA_5	GA_9	GA_1	GA_4	GA_8	GA_{13}	Allog.
Fragment ion	Possible Assignment	A	364	348	348	334	366	350	382	378	302
A-17	NH_3		✓	✓	B	B	B	B	B	B	B
A-35	NH_3, H_2O		B	B	✓	✓	✓	✓	✓	✓	✓
A-53	NH_3, $2H_2O$		✓	✓							
A-63	NH_3, HCOOH or H_2O, -COOH				✓	✓	✓	✓	✓	✓	✓
A-76	NH_3, C_3H_7O or H_2O, C_3H_6O										✓
A-79	NH_3, H_2O, CO_2		✓	✓							
A-81	NH_3, H_2O, HCOOH or $2H_2O$, -COOH		✓	✓			✓	✓	✓		
A-126	NH_3, H_2O, HCOOH, -COOH or $2H_2O$, CO_2, HCOOH		✓	✓							

A ≡ Gibberellin/Ammonium Adduct ion $(GA + NH_4)^+$ B ≡ Base peak of the spectrum

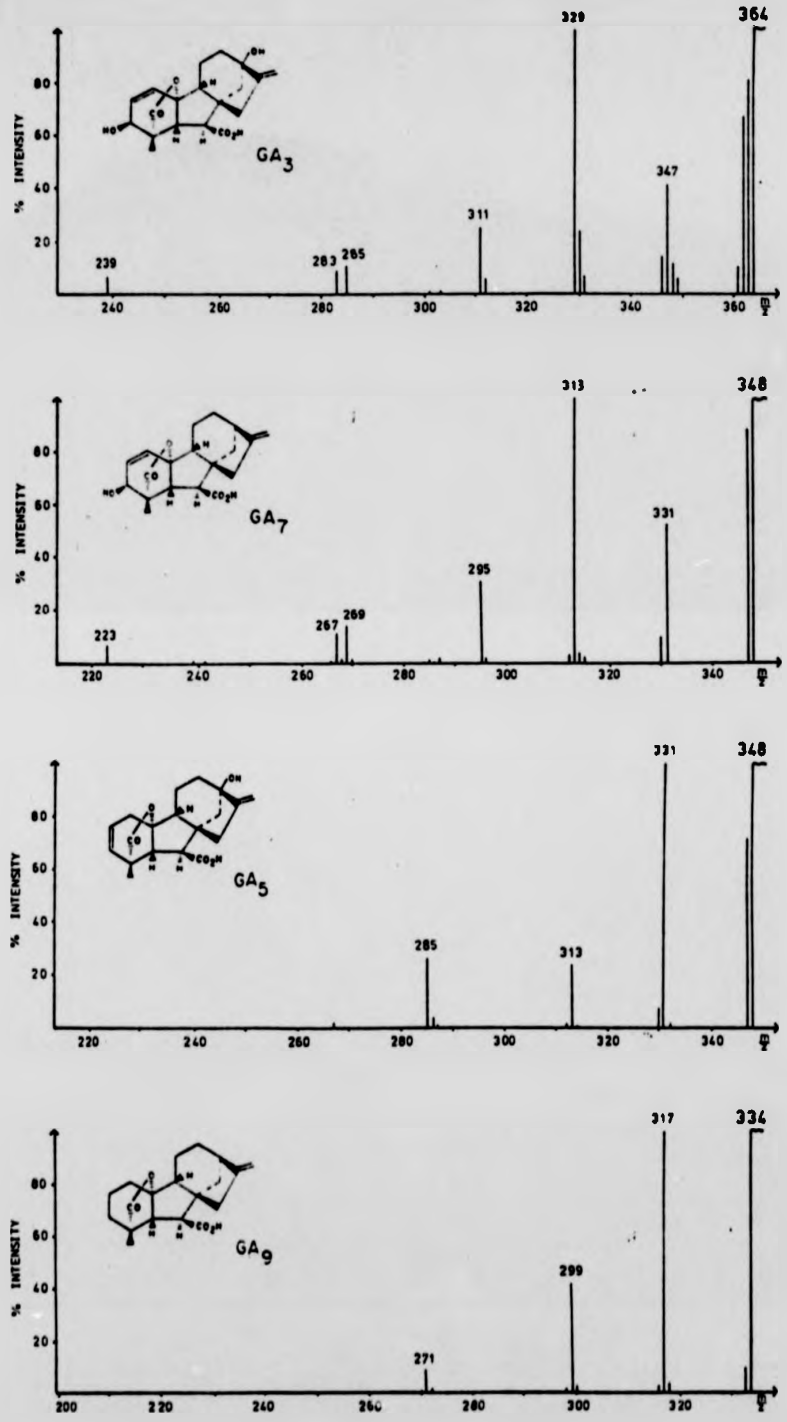

Figure 6.14. Collision induced decomposition spectra of some gibberellin/ammonium adduct ions. (GA_3, GA_7, GA_5 and GA_9)

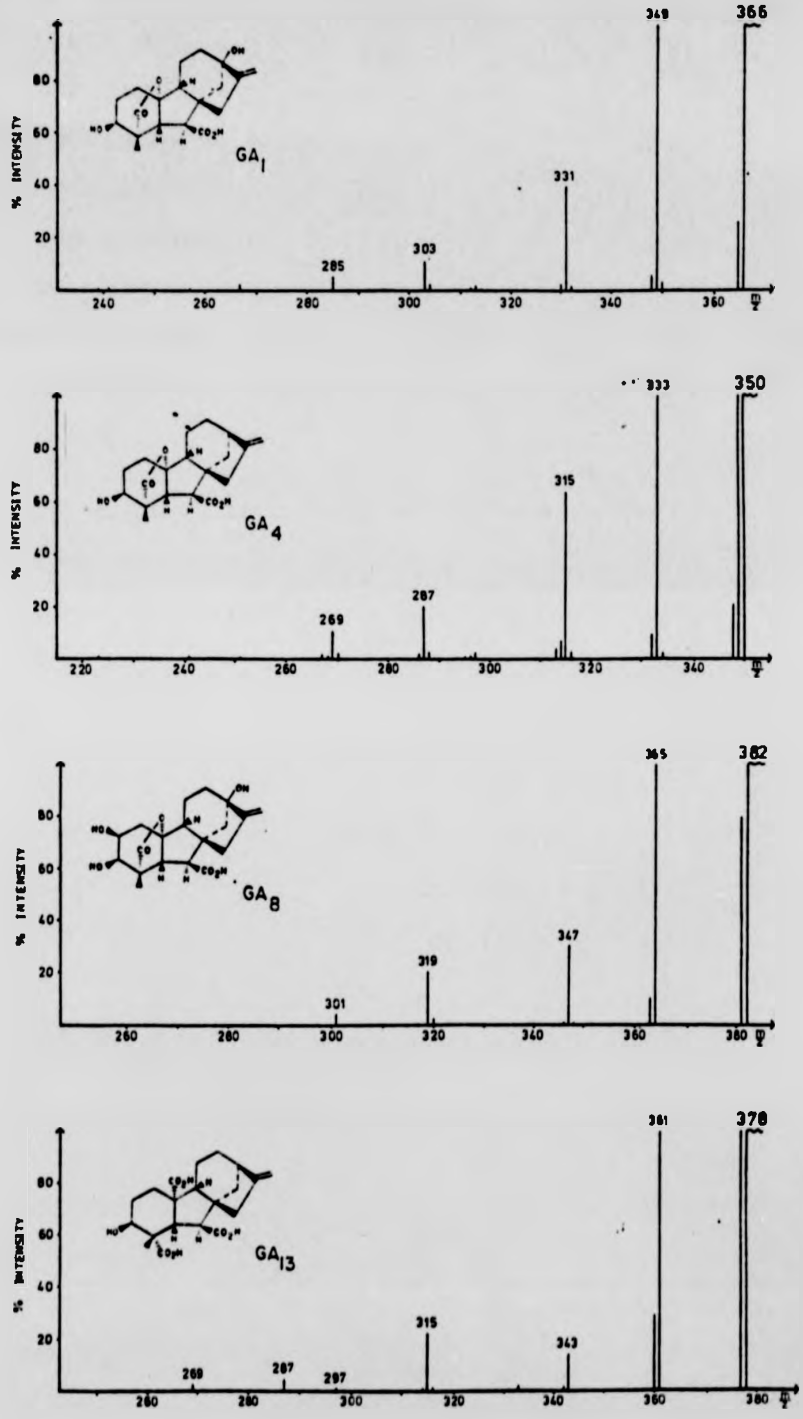

Figure 6.15. Collision induced decomposition spectra of some gibberellin/ammonium adduct ions. (GA_1, GA_4, GA_8 and GA_{13})

6.15, and examination of Table 6.2 allow this to be seen more clearly. The first column of this table lists the major (≥ 10%) fragment ions observed. Column two indicates the likely elemental composition of the neutral species lost in the fragmentation. The higher mass losses have been represented here, for the sake of convenience, as the sum of the loss of NH_3 and smaller neutral or radical species. It is not suggested that these are necessarily consecutive losses; indeed accurate mass measurements are required to confirm the total elemental composition in each case.

The analytical implications of these spectra are clear. All the B/E linked scan spectra are different either in terms of the pattern of the fragment peaks observed or the mass of the fragment peaks observed or both. Thus all the gibberellins examined can be distinguished easily.

Nine of the possible fifty-six GA structures have been examined so far and various structural trends are already evident. Thus a rationalisation of the linked scan spectra has been attempted by means of a correlation between the structure of the ion and the *pattern* of its B/E spectrum.

A metastable peak corresponding to the loss of ammonia from the $(GA + NH_4)^+$ ion is frequently observed on the x 100 scale in the conventional GA/ammonia chemical ionisation spectrum. Therefore it is not surprising that the loss of ammonia, giving an M-17 peak, generally forms the base peak of most of the B/E spectra. The two exceptions to this involve GA_3 and GA_7, whose base peaks are at M-35 (see Table 6.2). Several other similarities in the B/E spectra of GA_3 and GA_7 (Figure 6.13) allow them to be distinguished from other spectra. For example, the third most intense peak occurs at M-53 in contrast to M-63, as observed in all the other spectra. These spectra (GA_3 and GA_7) are also

the only two that exhibit the two peaks of approximately equal intensity at M-79 and M-81. Examination of the structures of GA_3 and GA_7 reveals that they differ from each other only in the presence (GA_3) or absence (GA_7) of a hydroxyl substituent at the junction of rings C and D. On the other hand, they both differ from all the other gibberellins examined in the structure of their A ring.

The usefulness of this ability to differentiate between different structures is further exemplified by a comparison of the B/E spectra of GA_7 and GA_5. They both have the same molecular weight and the same elemental compositions but the B/E spectra of their respective ammonium adduct ions are radically different (Figure 6.14).

The patterns of the B/E spectra of GA_5, GA_9, GA_1, GA_4 and GA_8 are less easily categorised because of their relative similarity. There are small differences which may be indicative of, for instance, the presence or not of unsaturation or the presence or not of a hydroxyl substituent on the A ring. For example, GA_1 and GA_4 both have the same A ring structure and similar B/E spectra; on the other hand the spectral pattern of GA_1 and GA_5 are different (see Figures 6.14 and 6.15).

This apparent dependence of the B/E spectra upon the structure of the A ring could indicate that this ring is the major site of ammination. It is a likely place because of the high electron density of this region owing to the presence of oxygen containing substituents (see Section 2.2.2).

An interesting case to test this hypothesis is posed by allogibberic acid whose structure does not contain any hydroxyl or lactone groups associated with the A ring. The appearance of m/z 226 (M-76) in both the B/E spectrum of the allogibberic acid/NH_4^+ ion (Figure 6.12(b)) and the e.i. spectrum of allogibberic acid may indicate preferential ammination in the C/D ring of allogibberic acid, involving the hydroxyl substitutent. (The formation of the ion of m/z 266 in the electron

impact spectrum has been attributed to the loss of C_3H_6O, Figure 6.7, involving this hydroxyl substituent and the D-ring carbon skeleton.[179]).

One theory that cannot be ruled out is the possibility that the carboxylic acid group, situated on ring B and common to all the structures examined, is a major site of ammination. The fragmentation of the ion is then directed from this site by the movement of electrons to stabilise the positive charge.

Finally, it should be remembered that GA_{13} has a tendency to pyrolyse (Section 6.3.3). Since the structure of the pyrolysis product is unknown, the structure of the ion of m/z 378 is also unknown and hence the B/E spectrum of this ion cannot be considered in this rationalisation. This does not however invalidate its analytical use.

6.3.5 Mixture Analysis

Initial mixture analysis work inolved the use of methanolic grass extracts which had been artificially doped with gibberellins. This preliminary work was performed in order to test the feasibility of the method in terms of sensitivity, prior to the detection of gibberellins in natural mixtures.

Experiments showed that the amount of the particular gibberellin to be detected needed to be present on the probe at the 0.1 to 1 µg level in order to obtain a single identifiable B/E spectrum. Bearing this in mind, standard grass extract solutions, which were obtained as described in Section 6.2, were doped with GA_3 or GA_5 to give concentrations of gibberellin to plant material from 100,000 ppm to 1,000 ppm, whilst allowing the gibberellin sample size placed on the probe to be of the correct order of magnitude.

It was possible to obtain one full identifiable B/E spectrum of GA_5 at the 3,000 ppm level using 0.6 µg of GA_5 and 0.2 mg of plant

extract on the probe. Using GA_3 it was possible to go as low as 1,000 ppm using 0.2 µg of GA_3 and 0.2 mg of plant extract on the probe. Unspiked grass extracts were run as a control. The unspiked grass extract clearly showed an ion of m/z 348 which became a doublet when the GA_5/grass extract mixture was examined. The linked scan spectrum of the ion of m/z 348 present in the unspiked sample gave only one fragment peak at m/z 331 (M-17) and thus did not interfere unduly with the linked scan spectrum of the $(GA_5 + NH_4)^{+\cdot}$, m/z 348, ion.

6.4 CONCLUSIONS

The following conclusions can be drawn about the work that has been completed to date.

A source of error in the literature has been discovered with regard to the pyrolysis of GA_3. This illustrates that care must be exercised in the assignments of fragmentation pathways.

The gas phase ion chemistry of GA_3 has been studied using positive and negative reagent ions. Ammonia has been determined to be the best chemical ionisation reagent gas for gibberellins.

Conditions have been established for obtaining the best chemical ionisation spectra of the gibberellins. Although these conditions are pertin ent to the particular instrument used the general method employed is likely to apply to most instruments.

All the GAs examined, including isomers, have been shown to be readily distinguishable by their collision induced decomposition spectra which were obtained using the B/E linked scanning technique.

The limited experiments performed using mixtures have shown promising results, with levels of detection reaching 1,000 ppm. Although this is somewhat higher than G.C./M.S., where detection levels of

10 ppm are common, various small modifications to this MS/MS technique would considerably improve the results obtained. For example, working at a higher accelerating voltage, say 8 or 10 kV and the use of a modern chemical ionisation source should lower the limit of detection to 100 ppm level. It should be noted that the use of a direct probe inlet as opposed to a G.C. inlet allows a larger amount of material to be placed in the ion source. Finally, detection levels could be reduced further by the use of selected decomposition monitoring (compare with single ion monitoring[183] used in G.C./M.S.).

One problem that was not tackled in this work, but which would need to be solved in order to make MS/MS as good a technique as G.C./M.S. for gibberellin analysis, is that of quantitation.

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